Vol. 26 No. 3

July - September, 1960

THE BOTANICAL REVIEW

Interpreting Botanical Progress

MICRURGY AND THE PLANT CELL
E. M. Hildebrand

ABSORPTION OF WATER BY PLANTS
R. O. Slatyer

LIBERATION OF ORGANIC SUBSTANCES FROM HIGHER PLANTS AND THEIR ROLE IN THE SOIL SICKNESS PROBLEM
Horst Börner

FUTURE CONTENTS
ON INSIDE BACK COVER

Published Quarterly

Second class postage paid at Plainfield, New Jersey

Printed in U.S.A.

THE BOTANICAL REVIEW

Founded in 1935 by

HENRY ALLAN GLEASON and EDMUND H. FULLING

Managed, edited and published at
The New York Botanical Garden
by

EDMUND H. FULLING

Advisory Editors

PROF. CHESTER A. ARNOLD
University of Michigan

PROF. M. M. RHOADES
Indiana University

PROF. MYRON P. BACKUS
University of Wisconsin

PROF. RUSSELL B. STEVENS
George Washington University

PROF. HERBERT C. HANSON
Catholic University of America

PROF. CONWAY ZIRKLE
University of Pennsylvania

Annual subscription rate: continental U.S.A. and Canada, $7.50; elsewhere, $8. Price of single copies of this issue: $2.

Information regarding availability and prices of volumes and single numbers previous to Vol. 25 will be furnished upon receipt of specific requests, but a list of every number in stock will not be supplied. Complete volumes and single numbers beginning with Vol. 25 are available at the prices stated on the inside front cover of each number.

Articles are obtained primarily by invitation. Unsolicited manuscripts are also welcome. In all cases, however, the editor reserves the right to accept, reject or suggest revisions in submitted material. All manuscripts should be on double-space typewritten pages, with references and citations in accordance with the customary style of THE BOTANICAL REVIEW.

Missing numbers can not be supplied without charge unless notice is received within two months after appearance of issues concerned.

All correspondence should be addressed to THE BOTANICAL REVIEW, NEW YORK BOTANICAL GARDEN, NEW YORK 58, N. Y.; not to Interstate Printing Corporation, Plainfield, N. J.

THE BOTANICAL REVIEW

VOL. 26 JULY-SEPTEMBER, 1960 NO. 3

MICRURGY AND THE PLANT CELL

E. M. HILDEBRAND

Crops Research Division,
Agricultural Research Service,
U. S. Department of Agriculture

Introduction 278
Micrurgical Research 279
The Science of Micrurgy 280
The Living Plant Cell 281
The Unit of Life 281
Components of the Cell 282
Physical Manipulation of Living Cells 283
Microinjection 283
Microdissection 283
The First Decade of Micrurgy 284
Physical Properties of Protoplasm 286
Structure and Viscosity 287
Wound Healing 288
Streaming of Protoplasm 290
The Plasma Membrane 291
Tonoplast-Vacuolar Membrane 292
The Cell Nucleus 293
Chromosomes 295
Plasmodesmata 296
The Cell Wall 297
The Electrokinetic Property 301
Miscellaneous Other Properties 303
Chambers, Dean of Micrurgists 304
Micrurgy and Phytopathology 304
Bacterial Investigations 305
Single-Cell Isolation 307
Single-Cell Inoculation 307
Single-Cell vs. Multicellular Inoculations with the Crown-Gall Organism 308
Virus Investigations 309
Intracellular Inclusions 311
Microdissection 312
Microinjection and Miscellany 315
Electron Microscopy 318
Fungus Investigations 319
Discussion 320
Literature Cited 321

INTRODUCTION

The title "Micrurgy and the Plant Cell" indicates the use of micromanipulation in the study of plant cells, in health and in disease. The title specifies the plant cell, whether in or of a uni- or multi-cellular organism. This review will examine the single-cell phase of host and parasite and also take into account the functioning of the plant cell and the host-parasite relationship in cytopathology. The science of micrurgy reveals the physical properties of normal-functioning cell components. It can be used in determining the specific number of pathogenic units for infectivity. Thus it clarifies the mode of infection at the basic level of cytopathology.

According to Chambers (1924), Hooke, Malpighi and Grew first recognized the existence of a viscid substance within living cells when they made their discoveries of the cellular structure of living things during the latter part of the 17th Century. Then a century passed before Corti and Treviranus observed streaming movements within plant cells, which led to a recognition of the fact that cell contents are essentially fluid.

The living substance of animal and plant cells was first correctly interpreted for animals by Dujardin (1835) and for plants by Von Mohl (1846). Dujardin proposed the term "sarcode" for the living material of "the foraminiferan body" in animal cells and of lower animals in general. He defined "sarcode" as "living jelly, gelatinous and transparent, insoluble in water, and capable of contraction into globular masses and of adhering to dissecting needles so that it can be drawn out like mucus". Von Mohl proposed the term "protoplasm" for the "slimy, granular semifluid" constituents of plant cells, which he distinguished from the cell wall, nucleus and cell sap.

Soon investigators identified Dujardin's animal sarcode with Von Mohl's plant protoplasm as the fundamental substance of the living cell. However, for a time their conclusions were lost sight of because of the more carefully worked out theory of Schleiden (1838) which relegated the seat of vital phenomena to the cell wall. It was through Schultze (1863) that protoplasm finally came to be regarded as the physical basis of life and that the universal occurrence and fundamental similarity of protoplasm in all living things became more widely recognized. In support of Schultze's work, Butschli (1876) maintained that protoplasm obeys the fundamental law of a fluid mass and performs life's functions as a fluid.

With protoplasm accepted as the seat of all life processes, speculative reasoning soon cast doubt upon the possibility of a liquid being able to manifest the variety of phenomena peculiar to life. Thus arose the "reticular" and "fibrillar" theories of protoplasmic structure that characterized protoplasm as a contractile solid of complicated organization containing a fluid in its interstices. Thus Chambers (1924) cited Heitzmann and Fromann among others who claimed that protoplasm possesses a delicate reticular structure, while Flemming (1891) and others considered that the substratum of protoplasm is essentially filamentous. These conceptions of visible structure were strengthened by cytologists who used "fixing agents" to coagulate protoplasm. Butschli (1876) and others argued that reticular and filamentous networks in protoplasm can be artifacts. Others mentioned by Chambers (1924) became convinced that protoplasm contains an "alveolar" or "foam" structure. The confusion was not settled by Altmann's "granular theory" that granules in hyaline protoplasm are invisible, in much the same way as colorless glass beads in oil are invisible. In the end, Butschli's alveolar theory was more widely accepted because of its close analogy to the structure of hydrophilic colloids.

To the following are attributed landmarks in search of greater precision and of the remarkable harmony of the principles resident in the Natural Law expressed by Virchow as "omnis cellula e cellula", by Flemming as "omnis nucleus e nucleo" and by DeVries as "omnis membrana e membrana".

Very little was actually known about the physical state of protoplasm until the present century with the advent of micromanipulation, for which Peterfi (1923) proposed the term "micrurgy". It was Chambers (1924) who pioneered the application of newer methods which clarified the misconceptions concerning the structure of protoplasm. Indeed, by direct micrurgical methods he demonstrated that animal protoplasm is a cellular unit which cannot exist without its nucleus and its cortex, and that is must be regarded not as a "stuff" but as a living mechanism consisting of visibly differentiated and essentially inter-related parts.

MICRURGICAL RESEARCH

Wright and McCoy (1927) improved the Chambers' micromanipulator for practical use. During 1927-1928 I undertook in Wright's laboratory an adaptation of Barber's (1914) single-cell technique for use with phytopathogenic bacteria, the organism causing crown gall

of fruit crop plants. At first, a single cell was drawn into the pipette tip, then transferred to liquid medium in a test tube by breaking off the tip with the contained cell and placing it in the medium. This technique always failed to result in growth of the single cell of the crown gall organism, regardless of the several ordinary and special media tried. The method was successful, however, with certain yeasts, with the pea blight organism, and a few others.

Further trials at culturing the single cell of the crown gall organism succeeded when the cell was placed in a micro-drop of ordinary medium in a hanging-drop mount. Viable cells multiplied promptly in the micro-drop (0.2 to 0.3 mm in diameter) and were ready within 24 hours for transfer to the test-tube culture. Deep-well slides designed to facilitate the handling of micro-drop cultures in that work have been available commercially for many years.

In 1939-1940 a Guggenheim Fellowship and the magnificent facilities of the Rockefeller Institute for Medical Research afforded me opportunity to extend my studies to the events involved in plant cell inoculation and infection with bacteria and viruses.

Further work was done in 1950 at the Marine Biological Laboratory at Woods Hole, Massachusetts, in association with Robert Chambers. There Parpart's television microscope was used in studies of protoplasmic streaming and of "X-bodies" and other inclusions in virus-infected cells; and, during various stages of plasmolysis, in observations of the tonoplast of pigmented cells of onion and of protoplasmic strands that pass through the plasmodesmata of contiguous host cells. Methods were sought for isolating uncontaminated cultures of algae. For further information on culturing algae, see Pringsheim (1946). The results of my studies have not been published.

THE SCIENCE OF MICRURGY

Barber (1904, 1908, 1911a, 1911b, 1914, 1920) gave birth to the new science of micrurgy when he invented the moist chamber (1904) in which to produce the microclimate essential for micromanipulation, specifically for the isolation of single bacterial cells. Next he (1908) invented a micromanipulator, a mechanical hand, for operating the micropipette under the highest powers of the microscope. Not the first such instrument to be invented, it was the first practical micrurgical instrument to be used by others (Hildebrand, 1938). This was followed

by pneumatic equipment for inoculating living plant cells with micro-organisms by micro-injection (1911, 1914). Barber's method supplied the essentials for micromanipulative or micrurgical work under the microscope: micropipette, moist chamber, micromanipulator, "hanging drop" working surface, proper light source and other requirements. Modified in several ways, his method for single-cell isolation is still perhaps the most used today.

In the first decade of his work on micro-dissection, Chambers (1918) employed Barber's micromanipulor and his method with a few modifications. The chief advantage of Barber's method over any other was in the "hanging drop" moist chamber. However, the microinjector was definitely lacking in quantitative control. Finding the micromanipulator inadequate because of mechanical difficulty, Chambers invented a new instrument, called the "Chambers' micromanipulator" (1921), which has not yet been excelled in precision. Two injection instruments were also devised (Taylor, 1920; Chambers, 1922). Another excellent micromanipulator was devised by Peterfi (1923). With these instruments, liquids can be driven through a micro-pipette with an opening only 0.5μ in diameter, and the amount of injection can instantly be controlled. These improvements inspired the most fruitful micro-dissection work of the present century.

THE LIVING PLANT CELL

THE UNIT OF LIFE

The cell or a unicellular organism is generally considered the unit of life. If the view is accepted that filterable viruses are living, then we must acknowledge that the lower limit of living bodies or entities approaches colloidal or molecular dimensions. This view would bear out the micellar hypothesis, in which the ultimate unit of life is regarded as a micelle, possibly of enzymatic nature, and protoplasm as an aggregate of living micellae. However, the micellar hypothesis is still only a speculation.

Cells are structural units of living matter, but in multicellular organisms they are subordinated functionally to the general economy of the organism as a whole. During the developmental period of a multicellular organism, the constituent cells undergo differentiation and become highly specialized in structure and function.

The protoplasm of a cell should be regarded not as a substance but as an organized physico-chemical system. In plant cells with prominent

cell walls, the protoplasmic body is called the "protoplast," a term which connotes an organized living unit. A significant feature of the protoplast, or protoplasmic body, is its microscopical dynamics, which were first made real and understandable through micrurgy.

COMPONENTS OF THE CELL

In a study of the physical constitution of protoplasm it is necessary to ascertain the extent to which protoplasm is normally enclosed within extraneous and apparently non-living coatings. In plants the protoplast is enclosed within a cell wall, from which it can usually be detached and still remain alive. In animal cells the existence of an extraneous coating is less obvious; nevertheless such a coating is probably an almost universal feature. The cellulose walls of plant cells and protein-like coatings of animal cells can be regarded as secretion products of the cells.

The term "plasma membrane" was used first by Pfeffer (1891) for the external surface layer of protoplasm to account for the results obtained in his classical studies on the osmotic properties of plant cells. During metabolic activity of cells the production of acid has no effect on the nuclear or cytoplasmic pH, but it may have a profound effect on the pH of the vacuoles and of the medium surrounding the cells.

Membranous structures are of great functional importance in protoplasm. The plasma membrane maintains the integrity of the protoplasm. Another membrane bounds the nucleus. Its permeability is quite different from that of the plasma membrane. A third membrane surrounds each of the vacuoles which are often very conspicuous in plant cells.

Granules are a common constituent of living cells. Fatty and crystalline bodies are also known to be present, but they most frequently appear enclosed within vacuoles. Mitochondria are minute, globular, rod or filamentous structures that appear to be universally present.

The nucleus is well recognized as the most significant constituent of protoplasm. Chromosomes, the bearers of hereditary characteristics, appear in the nucleus of cells preparing to divide, a fact well established by cytologists and geneticists.

An extensive method for studying permeability of functioning plant cells is the plasmolytic method which portrays the fact that protoplasm readily undergoes osmotic volume changes. Cells shrink or swell according to whether the suspension medium is hypertonic or hypotonic to them. Penetrability of a substance is revealed by the extent and

rate at which the shrunken protoplasts regain their original dimensions through deplasmolysis. Overton (1900) likened the plasma membrane to a lecithin or cholesterol mixture in an organic solvent.

The science of micrurgy created a new outlook and approach, in which midget tools are used under the microscope to delve into the fascinating worlds of the infinitely small (Battista, 1947). The micrurgical approach is by the most direct method. It employs single living cells of dimensions suitable for the purpose.

This review is a brief outline of what micrurgy has achieved in the specific area of the plant cell in the biological world.

PHYSICAL MANIPULATION OF LIVING CELLS

MICROINJECTION

Micrurgy afforded the means for the physical manipulation of living cells. Barber (1911, 1914) was the first to develop a practical technique for injecting bacteria and other substances into living animal and plant cells. Algae *(Nitella, Vaucheria)* and fungi *(Achlya, Dictyuchus, Saprolegnia)* were inoculated with vegetative bacteria, spores of bacteria, fungus spores, yeasts and other substances. The dosages varied from a few organisms to hundreds, and the organisms used for inocula *(Bacillus prodigiosus, B. typhosus, B. subtilis, B. coli-communis,* water bacteria, yeast, *Sporotrichum,* etc) found a good medium for growth in the interior of certain living plant cells. No evidence of antibacterial property was observed in the plant cells; and death of plants appeared due to parasitism, not to toxins. The host cells seemed to suffer little or no injury from being punctured. However, *Spirogyra,* one algal plant, appeared somewhat sensitive to injury.

MICRODISSECTION

Kite (1912) studied the structural components of protoplasm and noted that they vary greatly in their permeability to water, dyes and crystalloids. The rate of penetration of protoplasm by "dyes and crystalloids" appeared inversely proportional to the concentration of the living gel. Also the cell-walls, not the protoplasm, of many plant cells seemed to prevent the entrance of dyes.

Kite and Chambers (1912), considered the pioneer microdissectionists, made the first study on living chromosomes in cells of squash bug,

grasshoppers and crickets (because of their large cells and the clearness of nuclear figures).

Kite (1913) observed the physical properties of cells of certain animals and plants, including *Spirogyra,* using Barber's micromanipulator. He concluded that living matter occupies an intermediate position between true solids and true liquids, that it belongs to a class of colloids known as "emulsoids" and that it exists in either a gel (hydrogel) or sol (hydrosol) state. All the acid dyes tested entered *Spirogyra, Elodea, Hydrodictyon,* root hairs and parenchyma cells of *Tradescantia* when an extremely small puncture was made. The physical properties tested were rigidity, viscosity, glutinicity, elasticity, tenacity and colloidal state.

Kite (1915) in studies on the internal cytoplasm of plant and animal cells, produced artificial vacuoles within protoplasm by microinjection of some aqueous solutions but not with others because the formation of a bounding film depends both upon the solution injected and the way it is injected.

THE FIRST DECADE OF MICRURGY

Chambers (1915) summarized the first four years of microdissection studies by himself and Kite (Kite, 1912, 1913, 1915; Kite and Chambers, 1912; Chambers, 1914, 1915a). To Barber he attributed the opening up of a new field of endeavor, the micrurgical study of the physical manipulation and properties of protoplasm and of cell structures. He described and illustrated the apparatus used in cell dissection by going into minute detail on how to carry on dissection under the highest magnification of the microscope. Quotations from Chambers' classical report follow: "Protoplasm which is intermediate between true solids and true liquids has many properties of both. It may be described as an emulsion colloid, consisting of aggregates of albuminous molecules dispersed throughout an aqueous medium in which electrolytes and non-electrolytes are held in solution. This substance is homogeneous to the eye and may be liquid or solid according to the greater or less amount of the contained water".

"As we usually see it in living cells, the protoplasm is full of appreciable granules of varying sizes and droplets of apparently non-miscible fluids. As one watches them, the granules often coalesce or are drawn out into threadlets which may be crooked or straight, curled

or lumpy. Generally speaking, none of these are persistent; they disappear and reappear and can be interpreted as localized changes in the physical states of the colloids composing the protoplasm".

Extreme variability was observed in the consistency of protoplasm. Tearing may cause dissolution of the cell substance, setting free the nucleus and the protoplasmic granules. The soma of plant root-tip cells were found to consist of fairly rigid protoplasm. Pieces may be torn away without causing immediate dissolution. It is significant that a widespread result of mechanical injury to animal and plant cells is a gradual swelling.

The nucleus is a constant or essential component structure of the cell. In a normal living cell the nucleus tends to be an optically hyaline sphere, and dissection shows it to consist of a surprisingly more rigid gelatinous substance than the cytoplasm in which it lies.

"*Chromosomes.*—Upon more or less extensive injury to the cell the hyaline substance of the nucleus gives evidence of gradually increasing condensation or separating out of granules which collect to form an extensive network throughout the nucleus. In the germ cell of insects, where the cell and its nucleus are very large and favorable for study, this phenomenon is most interesting, for the granules arrange themselves into a constant number of filaments which shorten and go through all the changes in shape and size that have been described in the examination of chromosomes in fixed material. These artificially induced chromosomes, as also the chromosomes of normally dividing cells, may be pulled out of the cell and are found to possess the properties of a highly viscous and glutinous gel, readily swelling in water. The glutinosity of the chromosomes has been several times strikingly demonstrated when mobile spermatozoa are present in the dissection medium. A sperm will often strike against an isolated chromosome, which at once sticks to its tail and the sperm may be observed twirling away, dragging the chromosome behind it." . . . "In the dividing cell a marked bipolarity exists, the line connecting the two poles being at right angles to the plane of the future division. All structures in the cell at this stage tend to be stretched along lines which converge at the two poles, or so-called attraction centers. As the cell lengthens the cell structures become arranged in the form of a spindle, which is transformed into an hour-glass shape as a constriction midway between the poles, deepens around the cell. During this stage, tearing or puncturing the cell causes a loss of the polarity and the cell structures

wrinkle as the constriction disappears and the cell assumes a spherical shape". . . . "A feature of the dividing spindle is the appearance of fibers running from the chromosomes in the so-called equator and converging at the two opposite poles. These fibers have been considered as connected in some way with the migration of the chromosomes from the equator to the poles. In the germ cells of certain insects, such fibers have been dissected out of the cell. They are fairly resistant and are joined to the chromosome but not to the poles of the spindles. They seem to be pseudopodial prolongations of the equatorial chromosomes stretching toward the poles".

"In certain forms the microdissection method gave evidence of no spindle fibers whatever. In these cases the chromosomes lie in the equator imbedded in a hyaline gelatinous spindle-shaped mass. In fixed material this mass coagulates in the form of strands running from pole to pole, giving it the false appearance of consisting of threads".

"A common feature of all cell structures so far studied is that they swell and sooner or later go into solution when dissected out of the cell. Rigid structures at first become markedly glutinous and then gradually lose their viscosity as dissolution progresses. The structures appear to be due to different physical states of the colloids composing the protoplasm of the cell. The living cell may thus be defined as a mass of colloidal protoplasm in which the colloids may exist more or less permanently in different physical states".

PHYSICAL PROPERTIES OF PROTOPLASM

With a few exceptions, e.g., the gigantic cells of *Nitella,* protoplasm exists only in microscopic units of structure. Micro-manipulative operations must therefore be done in the field of a compound microscope by micrurgical methods. Von Mohl (1846) was the first to observe that protoplasm increases in viscosity with age. The cytoplasm of plant cells is distinguished and characterized by highly specialized bodies, the plastids, with synthetic functions that set them apart from animal cells.

Kite (1913) was the first to examine micrurgically the physical properties of plant cells and concluded that living cellular matter belongs to a class of colloids known as "emulsoids". Heilbrunn (1915, 1920, 1921), by employing the centrifuge method, proved that internal viscosity of gametes of dividing animal cells increases after fertilization.

STRUCTURE AND VISCOSITY

Seifriz (1918, 1920) made studies of the structure and viscosity of protoplasm in microscopic plants and animals as determined by microdissection by a modified Barber apparatus. He found that the consistency of protoplasm of a Myxomycete, when in the vegetative state, approached a liquid, while the density of the quiescent plasmodium approached the consistency of a gel. The quiescent protoplasmic mass possessed a somewhat plastic quality resembling bread dough. As a myxomycete prepares to fruit, the protoplasm increases in viscosity until it is of gel consistency. A tear in the highly viscous, inert plasmodial mass caused the formation of a rapidly enlarging protrusion and this flow of translucent liquid had its source within the protoplasmic mass. This substance seemed to be the matrix in which the granules were imbedded or the enchylema (interstitial substance), since the protoplasm was in the gel state and probably of sponge structure.

Seifriz found the protoplasm in the hyphae of bread mold *(Rhizopus nigricans)* in the quiescent state to be of very high consistency, sticky, quite elastic and very extensile, also closely resembling bread dough. When a filament of bread mold was torn, the inactive protoplasm did not flow out, but when pressure was exerted by a needle at some distance back from the torn end, the gelled protoplasm was forced out in the form of a rod which retained its shape until distrubed. The streaming protoplasm of *Rhizopus* was considerably less viscous than the quiescent protoplasm but not of such low viscosity as that in an active amoeba. Increase and decrease in viscosity were considered as probably dehydration and hydration phenomena.

While working with Hofler, Seifriz (1928) reported the onion *(Allium cepa)* as excellent material for microdissection. The purplish-red varieties are preferable because the color, being in the vacuole, does not interfere with observations on the surrounding transparent protoplasm. Seifriz described a technique for complete and clean stripping off of the entire protoplasm of an isolated protoplast from the vacuole, leaving the latter a free floating sac.

Seifriz (1929) gave an excellent review on the viscosity of protoplasm, with 142 literature citations. Discussing viscosity versus plasticity, he doubted that Poiseuille's law truly expresses the coefficient of viscosity because elastic liquids are plastic and not viscous.

Wada (1930) made a micrurgical study of the protoplasm in the hair cells of *Tradescantia virginiana.*

Seifriz (1930) noted that typical Butschlian alveolar protoplasm is characteristic of the ciliate Euplotes and other microscopic animals. Also, true alveoli are essentially vacuoles containing both inorganic and organic matter in solution. Pseudo-alveoli may be fat globules, vesicles or like bodies.

Scarth (1923) employed microdissection in studying various aspects of the protoplasm of *Spirogyra.* When using trivalent salts of alkalis in protoplasmic experiments he discovered that the protoplast failed to separate from the cell wall except at the ends. Scarth likened the adhesion of the protoplast to the cell wall to other phenomena such as adhesion of leucocyte to bacterium in phagocytosis.

Normally chloroplasts of *Spirogyra* (Scarth 1924a) are in the gel state and appear to be elastic. Under the action of certain stimuli they undergo longitudinal, reversible contractions termed "vital". This leads to solation of the gel which then behaves like a fluid body in "disintegrative" contraction. The agents that produce "vital" contraction are always capable of producing solation. During contraction chloroplasts apparently have no intake of water, but there is an output. The contractile organ is essentially an optically void emulsoid in both the gel and the sol state.

Scarth (1924b) observed that under the action of cations protoplasm resembles a physical colloid, e.g., contraction and solation of a gel, or a fall in viscosity of a sol. In both cases the general order of efficiency of the various classes of ions is similar. The more powerful ions exert their maximum effect at intermediate concentrations. As a rule, typical "vital" or "disintegrative" contraction happens within an hour or not at all.

In another report, Scarth (1927) studied the structural organization of plant protoplasm. The fluidity observed through the microscope was illusory, and cytoplasm was always more or less elastic, even when streaming. Although denying the existence of a persistent framework to protoplasm, the resting nucleus seemed to possess structure.

WOUND HEALING

Topler (1903), in some experiments on injured cells of *Bornetia secundiflora,* found that the protoplasm remaining in an injured cell would form a new wall. In his experiments the amount of protoplasm lost was sufficient to cause the remainder to contract from the old

wall against the adjacent uninjured cell of the filament, where it sometimes assumed an abnormal shape. A new wall was then developed by the protoplasm, forming a new cell.

Nichols (1922) studied some of the reactions of mature plant cells when punctured by a sharp needle and noted: *a)* all but one of the algae studied were able to heal a wound; *b)* the density of the protoplasm varied from a very liquid condition in some species to a quite viscid condition in others; *c)* no correlation was found between the density of the protoplasm and the character of the cell wall; *d)* the exuded protoplasm may or may not be miscible with water; *e)* if the exuded protoplasm is non-miscible, the film formed over the escaping protoplasm is not comparable with the plasma membrane; *f)* the puncture is not closed by a film or membrane but by an accumulation of plastids, pyrenoids and starch granules in the opening. When a Spirogyro cell was punctured and the single nucleus removed, no recovery followed, even though the hole was small and the protoplasmic loss slight.

Nichols (1925) studied the effect of wounds upon the rotation of the protoplasm in the internodes of *Nitella*. A portion of a plant two or three internodes long was mounted in a drop of water under a microscope with a low-power objective and punctured to produce traumatic shock. When the needle is slightly dull the wall bends at the point of pressure and, as the needle penetrates, the wall springs back into position, causing a slight jar or vibration to pass through the cell. This action is accompanied by an immediate cessation of movement throughout the cell. Complete cessation was never observed except when it could be accounted for by either readjustment of the wall or an explosive loss of protoplasm following the puncture. That a jar or a mechanical shock may start a vibration which will cause cessation throughout a streaming cell has been observed so many times that it does not require further comment here.

When a slight wound was made, some of the particles in the current moving away from the injury ceased to move immediately while others continued to move slowly (80μ per 52 seconds). A little later some granules were observed moving 80 microns in 20 seconds. Next morning the entire protoplasm of the cell was moving or rotating normally at 80 microns per 1.5 seconds. Thus wounding produces a shock which interferes with the streaming of protoplasm.

STREAMING OF PROTOPLASM

Movement in protoplasm has been recorded since the earliest days of the compound microscope. Movement is one of the basic criteria of life. Many years ago Schultze (1863) and Kuhne (1864) studied the effect of a variety of agents on streaming protoplasm. According to Schultze, an increase in temperature increased streaming within limits. Kuhne found the same true for temperature changes in either direction (an irritability effect). He noted that movement ceases when streaming protoplasm is exposed to induction currents, water vapor, ether, chloroform, extremes in temperature, or inclosure in a layer of an indifferent oil. When the return to normal conditions started streaming again, Kuhne ascribed the cessation to lack of oxygen.

Nichols (1925), in observations on the rotation of protoplasm in *Nitella,* noted complete cessation at the wound, but the next morning recorded a speed of 80 μ per 1.5 seconds after complete recovery from traumatic shock. Peterfi (1927) applied the term "thixotropy" to a colloid which when stirred is caused to flow by changing from a jelly to a liquid.

In many plant protoplasts (Chambers, 1935) fluidity of protoplasm is evident from the continuous streaming exhibited in cyclosis. The existence of "Brownian movement" made visible by dark ground illumination appears to be almost a universal phenomenon in animal protoplasm. However, in plant cells "Brownian movement" has been detected only in localized regions of cytoplasm. This movement is usually observed not only in the more fluid regions of animal cells but also in regions which microdissecting needles have shown to be in a jellied state. A remarkable feature is the fact that semisolid regions in protoplasm can sometimes be made suddenly fluid by mechanical agitation.

Buller (1931, 1933) made a thorough study of hyphal fusions and protoplasmic streaming in the higher fungi. He noted that protoplasm of *Coprinus* was in movement in the process of forming the sexual fruiting body. Translocation of protoplasm from the old to the younger hyphae was by protoplasmic streaming. The old large vacuoles and most of the old small ones remain fixed to cell walls. Young or small vacuoles and most other particles move. Young small vacuoles may be carried in the streaming for long distances, passing through pores in the septa of the end walls of cells. The traveling of the vacuoles was

followed in numerous specimens. In *Fimetaria fimicola* the average speed was .01 mm. per second.

The direction of the protoplasmic current was observed in numerous specimens. Movement occurred in one direction only without any reversal, being always from old hyphae which had ceased to grow toward rapidly growing younger ones. The hyphae which receive nutriment from the mycelium by the copious streaming of the protoplasm play no small role in the life of the fungus. The causes of streaming appear two-fold—vacuolar pressure and increase in amount of protoplasm through absorption. No doubt vacuolar pressure would arise at least in part from the latter.

Studying *Pyronema confluens* and using a fine needle for wounding, Buller found that the protoplasm of a cell injured by the needle always died throughout the length of the cell and up to its two septae. The injury was not transmitted to the two adjacent cells which remained living. Two things happened immediately after a cell was killed: *a)* the septae separating the dead cell from the two adjacent living cells became convexo-concave, with the convex side toward the lumen of the dead cell; *b)* pores of the two septae became blocked by protoplasmic plugs which prevented escape of living protoplasm from the contiguous living cells into the dead cell.

In the Mucorineae of the Phycomycetes, protoplasmic streaming is characterized by *a)* frequent reversal in direction of flow, *b)* peripheral current of protoplasm flowing opposite to main stream, and *c)* general central movement of vacuoles.

THE PLASMA MEMBRANE

The plasma membrane is an organized living structure surrounding the protoplast. Its semi-permeability is a function of its surface which is made up of passive and physico-chemically active parts in combination.

Overton (1900) proposed the "lipoid theory of the cell membrane" within which the protoplasm maintains itself in an aqueous environment.

Pfeffer (1890) considered the cell membrane responsible for the semi-permeable properties of protoplasm. In his "plasma membrane theory" he assumed the surface of protoplasm to be an osmotic film one to two molecules thick and consisting mainly of proteins. Im-

mersing a living cell in a weak solution of HCl caused the plasma membrane to assume the condition of rigor without destroying its semi-permeability. Under these conditions (Chambers, 1924) a dye which does not penetrate the surface membrane will enter through a tear and rapidly diffuse throughout the interior of the cell. Pfeffer showed that formation of surface films is an innate property of the protoplasm, and these films can be readily produced de novo. One of his experiments consisted of introducing asparagin and gypsum crystals into the body of a myxomycete plasmodium. Imbedded in protoplasm, the crystals gradually imbibed water and formed vacuoles bounded by distinct surface films. Listei (1888) obtained clear protoplasm by filtering out spores of the myxomycete *Badhamia* through wet cotton.

TONOPLAST-VACUOLAR MEMBRANE

The usual method of obtaining tonoplasts is that originally used by DeVries, namely, subjecting pieces of plant tissue to the action of hypertonic solutions of a potassium salt. DeVries applied the term "tonoplast" strictly to the wall or enclosing membrane of the vacuole. The nature of the tonoplastic membrane after plasmolysis or death of the protoplast has been the subject of dispute. Some consider this membrane a coagulation product of a protein nature. Others regard it as an actual inner plasma membrane which has separated from the protoplast at the death of the latter.

Hofler began his micrurgical investigations on plasmolyzed onion cells in 1927. The instrument used was a Zeiss-Peterfi micromanipulator. In June Hofler was joined by Seifriz. The plant used was the onion *(Allium cepa)*, preferably a purplish red bulb variety in which the color is in the vacuole and does not interfere with observations in the transparent cytoplasm. Seifriz (1928) reported the onion an excellent material for microdissection. The technique consisted in slicing off a small piece of the lower epidermis and plasmolyzing it in hypertonic solution (0.8 M KCl). The thin slice was then cut transversely with a sharp razor, leaving open at the cut edge several cells, the plasmolyzed protoplasts of which had not been touched. These open protoplasts afford ready entrance for a needle. One startling observation was that of complete and clean stripping off of the entire protoplasm of an isolated protoplast from the vacuole, leaving the latter a free floating sac, which was illustrated.

Chambers and Hofler (1931) made further studies on the tonoplast

of *Allium cepa*. They extended the term "tonoplast" to the body of the vacuole as a whole with its investing membrane. With glass needles cytoplasmic strands of cohesive, fluid material can be pulled out from the surface of protoplasts plasmolyzed in 1.2 M glucose, and when released they flow back. If they are kept extended their material collects into ever enlarging beads along the length of the strands. A drastic tear with needles causes the cytoplasm to change from a glassy, translucent to an irregular, granular aspect and to degenerate into a glutinous mass of variable sized vesicles. The nucleus of a protoplast in KCl solution is a swollen sphere which disappears when punctured. An intact protoplast removed from its cell into 1.2 M sugar solution gradually assumes a spherical shape and falls of its own weight to the bottom of the hanging drop and ruptures.

Naked tonoplasts were obtained from the epidermal cells of *Allium* and treated with 0.8 M KCl or KBr and 1.2 M glucose. The tonoplasts were easily freed of extraneous material by picking it away with microneedles. Naked tonoplasts in sugar solutions were obtained directly from living protoplasts by tearing away the protoplasm. The membrane of the naked tonoplast appears to be a highly cohesive and extensible fluid film of unappreciable thickness. Its cohesiveness is such that a glass microneedle can be passed readily through it from one side to the other without causing rupture. The membrane simply closes over the moving needle and remains intact. Upon adhesion of a tonoplastic membrane to a glass needle, delicate strands can be pulled out which, when released, flow back into the membrane. The highly fluid nature of the membrane is a striking characteristic. A tonoplast can be distended considerably by injecting water or sugar solution. A small air bubble adheres slightly to the surface of a tonoplast. A droplet of liquid paraffin or olive oil snaps on and adheres so strongly that it cannot be removed without rupturing the membrane or carrying a portion of tonoplast away with it. A droplet of chloroform also adheres, but within a few seconds after contact it destroys the tonoplastic membrane. All evidence points to the conclusion that the tonoplast of an onion cell is bounded by a thin, liquid film consisting of material not miscible with water.

THE CELL NUCLEUS

The discovery that the nucleus contains chromosomes was made by Schneider in 1873 (Chambers, 1924). When the microdissection

method was first applied to study of the nucleus, Kite (1913) concluded that typical nucleus is a gel containing more concentrated areas (granules) and an important network. The difficulty in interpreting the physical structure of the nucleus is due to the extroadinary ease with which its substance sets into a relatively firm gel. This accords with the fact that one of the chemical constituents of nuclei is nucleic acid, which has remarkable powers of forming gels.

Sinke (1939) made experimental studies of cell nuclei, testing the effects of hypertonic and hypotonic solutions, high and low temperatures, acids and alkalis, neutral salts, chloral-hydrate and coal tar extract, effect of infections on lower organisms and effect of mechanical injury. The objects tested were epidermal and hair cells, animal cells, pollen mother cells of *Trillium*, guard cells of the stomata, and root tip cells of *Vicia*.

Chambers and Black (1941) tested electrolytes on the nuclear structure of the cells of onion-bulb epidermis. The criterion for viability was the persistence of cyclosis. They noted two types of visible structureless nuclei: *a)* vitreous nuclei, in which the entire nucleus appears homogeneous but highly refractive by contrast with the surrounding cytoplasm; *b)* phantom nuclei, in which the nuclei become hyaline and appear like an empty space bounded by cytoplasm except for the persisting nucleoli. In plasmolyzed cells vitreous nuclei are infrequent. Cyclosis of cells with vitreous nuclei is erratic or irreversibly stopped. Phantom nuclei appear in large numbers upon deplasmolysis of cells exposed, when plasmolyzed, to optimal concentrations of certain electrolytes. Leaf cells of *Tradescantia* differ from onion cells by losing structure when plasmolyzed in neutral sucrose solutions and regaining it when deplasmolyzed. In acid-sucrose solutions (pH 4.5-5.0) these cells, if plasmolyzed, develop phantom nuclei on deplasmolysis.

Chambers (1941) found that the pigment of normal, living cells of tulip petals is restricted to the sap in the cell vacuole. When the cells are crushed, the ensuing injury results in a breakdown of the cytoplasm and direct contact of the exposed nucleus to the pigment, which becomes preferentially absorbed by the nucleus and thereupon assumes the color of its extreme alkaline range. The natural pigments of tulip flower thus furnish an indicator by which conclusions from other sources regarding alkalinity of the nucleus as compared with its surroundings can be confirmed.

CHROMOSOMES

Kite and Chambers (1912) made the first micrurgical study of living chromosomes in testes of squash bugs, grasshoppers and crickets, which have very large cells and clear nuclear figures. In microdrops (in Barber's moist chamber) and stained with Janus green, the stained chromosomes continued to live. Transformations of anaphase figures to telophase were easily followed. Dissections were made of the colloidal structure of resting and dividing nuclei. Single chromosomes in the prophase, metaphase and telophase stages were dissected out of cells. The chromosome is a fairly concentrated and somewhat refractive gel. It varies in elasticity in its different phases. A single metaphase chromosome with its spindle fiber attached was dissected out. The spindle fiber is a slightly refractive elastic gel and in the metaphase seems to be continuous with the chromosome. The nuclear network (spireme, spindle, chromosome) is imbedded in a dilute gelatinous gel that is commonly invisible except when dyed.

Employing the new Chambers micromanipulator (Chambers, 1921, 1922), Chambers and Sands (1923) dissected the chromosomes in the pollen mother cells of *Tradescantia virginiana* with a bent tip micropipette. Mounted in equal parts of ten percent saccharose and sap of the cut ends of stalk, the pollen mother cells were dissected and the chromosomes isolated and studied. To illustrate, one chromosome is a definite ring with transverse constrictions; another is almost a closed U with one part on end viewed in optical section being distinctly a cylinder exhibiting a medulla, with a refractive index very different from that of its cortex. Plant chromosomes are very resistant to injury, whereas animal chromosomes (grasshopper) disintegrate soon after removal from the cell. The isolated chromosome is a body which can be stretched with needles and torn into stringy masses. Regarding the consistency and structure of the chromosome, the following conclusion was drawn: The metaphase chromosome is a gelatinous and extensile body which may give evidence of a definite structure in possessing a cortex that can be optically differentiated from a central core.

Sands (1923) observed the structure of the chromosomes of *Tradescantia virginiana* and made a distinction between chromatin and linin. The linin is distinctly a cylinder of jelly-like consistency in which the chromatin is imbedded. The chromatin is grouped into bodies, the generally recognized chromomeres. In *Tradescantia* these

are of variable shape and size, and their number in the chromosome seems variable. The colloidal structure appears to be primarily a two-phase system with the chromatin representing the disperse phase and the linin the continuous phase. At any particular stage of mitosis, an individual chromomere may have a greater or lesser degree of dehydration than its neighbors in the same or other chromosomes. The degree of dehydration may represent a specific constant for each chromomere and for any particular stage.

Sands (1925) made further observations on some aspects of mitosis in *Tradescantia virginiana* with emphasis on the pachytene threads. Micro-dissection of the pachytene threads shows that the relation of *Tradescantia* chromosomes is a matter of continuous linkage in a chain and that division and segregation are everywhere processes of abstriction with subsequent mechanical distribution of the elements. Linkage occurs because constrictions may lag or because abstriction may be suppressed, perhaps permanently in some cases. Since *Tradescantia* does not show a longitudinal split in prophase, use of the term "equatorial division" should be avoided except as it implies separation of sister chromosomes that have arisen by abstrictions in pairs (two univalents) from a continuous filament. Many counts show that the number of so-called pairs corresponds to half the number of somatic chromosomes.

PLASMODESMATA

Plasmodesmata are delicate threads of cytoplasm which connect the protoplasts of adjacent plant cells (Eames and MacDaniels, 1947). They are characteristic of living cells and undoubtedly are present in all living parts of higher plants, maintaining the continuity of the protoplasm of the plant, although its units, the cells, are set apart by firm walls. Their extreme delicacy and protoplasmic nature make special technique necessary for demonstration of them in most tissues. They are extremely fine in gymnosperms. They have not been demonstrated in the earliest stage of wall formation and disappear with death of the cell. In tracheids and vessel elements they disappear with the protoplast. They occur in groups and are scattered. Plasmodesmata probably represent persisting connections of the protoplasts of daughter cells, although their presence in the first stages of wall formation has not been demonstrated. The cytoplasmic connections become detectable as the cellulose and pectic materials are laid down in three layers between the two protoplasts. The number of plasmodesmata seems to

increase with growth of a cell. Their function may be as channels for translocation of solids and stimuli joining the protoplasts of the plant body into a single protoplasmic structure.

Scott (1948, 1950) noted suberin in parenchyma, sclerenchyma, phloem and xylem of vascular strands. The differentiating element begins to expand as soon as it is cut off from the parent cambial cell. At this early stage the wall is a cellulose net, the roughly hexagonal meshes of which are perforated by closely set plasmodesms, less than one μ in diameter. In the profile of the cell wall, the plasmodesms are observed connecting the protoplasts of the differentiating adjacent cells, vessel segments, fibers, xylem parenchyma or rays. Deposition of the secondary wall becomes marked when the vessel segment reaches its maximum diameter. About this time extracellular and intracellular suberin layers become visible. Extracellular suberization appears to begin at cell corners and extend therefrom along the middle lamella. As wall growth continues, the mouths of the pits are narrowed by overhang of cellwall, and sooner or later liquification of wall occurs. As end walls of vessel segment disappear and the protoplast eventually dies, the plasmodesms withdraw from pit areas, leaving behind a membrane that seals the base of the pit. This withdrawal explains the absence of plasmodesms in walls of liquified elements. The mature xylem vessel is thus completely lined throughout its length with a suberized membrane.

Scott (1949) distinguished and demonstrated plasmodesmata in differentiating and mature xylem vessels—spiral, reticulate and pitted—in the leaf of *Acanthus mollis*, the stem of *Echinocystis macrocarpa* and the root of *Vicia faba*. The ubiquitous occurrence of plasmodesmata is of undoubted significance in physiological problems related to movement of solutes in plants.

THE CELL WALL

Nageli (1858) developed the hypothesis that the plant-cell membrane, or wall, is composed of submicroscopic units—"micellae". According to Van Iterson (1927), a different principle underlies the submicroscopical structure of the secondary wall, that is, the direction of flow of the protoplasm depositing the laminae of apposition. He contended that currents of plasm can be observed to circulate, depositing rings or bars during vascular formation.

Thomas (1928) studied the composition of cell walls of 12 species

of *Fusarium* and found the same general structural plan in all with a basic skeleton of chitin. Thomas (1930) made a similar study on *Sclerotinia* and found that all six species had the same composition of cell structure because under the outer covering of callose was the basic skeleton of chitin.

Wall formation was first evident at the close of nuclear division (Eames and MacDaniels, 1925, 1947). The exact nature of the fluid, cell-plate material is not well defined. Norman (1937), in his book on the biochemistry of cellulose, etc., characterizes the cell wall of living and dead organic matter as forming the non-living record of some of the metabolic activities of the protoplasm, with the cytoplasm being primarily nitrogenous and the cell wall carbohydrate in nature. He states that the functions of the cell and the tissue where cellulose occurs will inevitably affect the composition of the wall, and yet these activities are not sufficiently comprehended to permit the history of the development of the cell wall to be written. The main structural constituents are both non-living and insoluble, and no enzymes capable of effecting the breakdown of the two important end-products, cellulose and lignin, have yet been detected. This deficiency calls for the micrurgical approach as the best hope for solution.

Farr and Eckerson (1934) separated cellulose particles in membranes of cotton fibers by treatment with hydrochloric acid. Removal of non-cellulosic substances from the cellulose particles in the living protoplasm in the mature cell wall was accomplished previously by means of mild pectic solvents. Microchemical, optical and X-ray diffraction analyses showed that the essential cellulose nature of the particles was unaltered.

Becker (1934) states that the cell plate in the phragmoplast of staminal hairs of *Tradescantia* first became visible as droplets exhibiting Brownian movement. He says that they do not move along the spindle filaments, as others assume, but are formed just where they are by dissociation from the dense plasm.

Farr (1934) explains that certain X-ray diffraction phenomena, which heretofore seemed to necessitate assumption of the existence of hypothetical sub-microscopic micellae, are explained equally well by the microscopically visible particles of cellulose, united in later stages of growth by cementing substances to form fibrils that mask the cellulose particles.

Cellulose membranes were defined by Farr (1937) as plant cell

walls, in which there are no appreciable accumulations of such materials as lignin and suberin. Their relation to the non-cellulosic constituents is highly variable. The membrane of this type consists of a dense, homogeneous, colloidal mass, in the inner regions of which are embedded numbers of uniform size, ellipsoid particles of cellulose. Surrounded by a thin layer of colloidal material, they occur separately in the cytoplasm of the young cotton fiber. Throughout the process of wall formation the relation between the cellulose particle and its colloidal coating remains unchanged.

In further observations Farr (1938) established that the cellulose particles were one μ in diameter and that the space between the crystallites is not vacant, as Nageli believed, but occupied by a colloidal material having physical and chemical properties very different from those of the cellulose particles. The presence of this colloidal matrix accounts for the unique swelling behavior of plant-cell membranes, or walls. This study demonstrated the remarkable uniformity in size, shape, physical and chemical properties of cellulose particles from all parts of the plant kingdom.

Farr and Sisson (1939) made observations on the membranes of epidermal cells of the *Avena* coleoptile during cell enlargement. The delicate membranes of very young epidermal cells of the coleoptile are composed of non-cellulosic material. Microchemical analyses show the presence of both pectic and wax-like material along with traces of the components of the original limiting membranes of the protoplast. During the early period of cell elongation, visible ellipsoid particles of cellulose accumulate in the outer cytoplasm and become oriented in fine transverse bands. At first the cellulose particles are arranged in a single row, side by side, and form a band held together by non-cellulosic material. As the elongation continues, the cellulose particles become farther apart but without change of orientation in the membrane. With time there follows one or more additional layers of cellulose. That the plastic state of the non-cellulosic component is maintained during the elongation period is shown by the separation of the transverse bands of cellulose particles. Thus the tension of growth is exerted not upon the cellulose but upon the more plastic, non-cellulosic continuous phase of the membrane (wall).

Eames and MacDaniels (1947) indicate that in the primary wall pectic substances accompany the cellulose in early stages. The secondary wall is at first composed mostly of cellulose or cellulose and hemi-

celluloses. Substances that later replace or are added to these are lignin, cutin, suberin, various inorganic materials, tannins, oils and other materials.

Frey-Wyssling (1948) investigated the submicroscopic morphology of protoplasm and its derivatives. He contends that there is a very real physiological and histochemical difference between the primary cell wall of vegetable meristems and the secondary membranes of grown tissue. The primary wall is deposited by intussusception. The secondary walls are deposited by apposition against the fully grown primary wall during the corresponding growth of the membrane in thickness. The primary walls lack the microscopic markings such as pits and striations. They are very thin and are considered as the intermediate membrane between neighboring cells. Actually though, the membrane consists of three lamellae—the original odd middle lamella produced from the cell plate during cell division and the two primary walls added on to it.

An important point about meristematic cell walls is that no cellulose can be identified microchemically in them. Birefringence enables the investigator to detect when, during formation of the young membrane after division of the cell, cellulose first makes its appearance. Frey-Wyssling illustrated the submicroscopic structural plan of the meristematic primary wall of cylindrical cells. Various other structural plans are illustrated, including one showing how cellulose strands are embedded by intussusception.

The coherent texture of interwoven micellar strands (with a diameter of 15-20μ (150-200 Å) has been demonstrated by Elvers (1943) with electron micrographs.

Wyckoff (1949) assembled valuable knowledge on techniques and applications of electron microscopy. Many particles of macromolecular dimensions in nature are rods or filaments. Cellulose has elementary particles of this sort and so apparently have the nucleic acids.

J. F. Scott (1948) made electron micrograph studies on sodium desoxyribose nucleate obtained from nucleoproteins. Underlying all is the fact that structures built of cellulose, regardless or origin, consist of elementary filaments about 30μ (300 Å) in diameter. This is true whether dealing with cellulose of bacterial origin (Franz et al, 1943), walls of simple plants such as *Valonia* (Preston et al., 1948), coleoptiles of sprouting seeds or fibers (Muhlethaler, 1949) of wood, cotton, hemp, flax and other industrially important plants. The char-

acteristic properties of cellulose from different sources are determined by the arrangement of these elementary fibrils and by the various amounts of other substances like hemicelluloses and lignin with which they are intimately associated. Before cellulose can be satisfactorily examined under the electron microscope, it must be freed from these impurities. This is not the case with the naturally clean fibrils of bacterial cellulose as are made by *Acetobacter xylinum* growing in liquid culture. Separate filaments as well as their simple associations in either twisted bundles or flat ribbons are clearly visible.

Green and Chapman (1955) studied, by means of the electron microscope, the development and structure of the cell wall of *Nitella*. The evenly distributed pattern of growth and twisting of the *Nitella* cell wall is most easily interpreted as the result of a "mosaic" type of wall development. Isolated small areas are presumed to grow intermittently in a manner evenly distributed throughout the wall. Allometric growth data indicate that each growth event in the mosaic pattern has the same ratio of percent increase in the transverse direction to the percent increase in the longitudinal direction. Structural investigations reveal that the cellulose microfibrils of the wall are well oriented and run strictly in the transverse direction. Thus the twisting or spiral growth of the wall is not related to a helical arrangement of the wall elements but to a lateral shifting of the transverse structural members that takes place in all portions of the wall so that the entire volume behaves as a unit.

THE ELECTROKINETIC PROPERTY

Determination of actual acidity or pH came into its own in the 1920's.

Needham and Needham (1925) used a specially constructed moist chamber suitable for studying pH and eH of the interiors of living cells in atmospheres of various gases. They found the cell interior of *Amoeba proteus* had a pH of approximately 7.6 and an O-R potential of between rH 17 and 19.

Scarth (1926) studied the influence of external osmotic pressure and of disturbance of the cell surface on the permeability of living cells of *Spirogyra* by acid dyes. The observed facts point to an organized film on the surface of the cytoplasm as the regulator of permeability.

Gelfan (1927) measured the electrical conductivity of the protoplasm of *Nitella* and also of several microscopic animals by inserting

non-polarizable microelectrodes of one to two μ diameter into the interior of the living cell. The specific conductivity, in reciprocal ohms at 18°C., was higher for *Nitella* than for the more active animals *(Paramecium,* etc.), suggesting that the concentration of dissociated molecules in the protoplasm is in some way related to the activity of the organism.

Taylor and Whitaker (1927) made potentiometric determinations in the protoplasm and cell sap of *Nitella* and found that these substances behaved very differently toward the hydrogen electrodes. The values for protoplasm, of between + .093 and + .030 volts, were interpreted as indicating eH or oxidation-reduction potential, not pH, while the values for the cell-sap approximated pH 6.0.

Chambers (1929a) observed production of acid by injured cytoplasm. For determining the hydrogen ion concentration of protoplasm, he tested the colorimetric method in three ways: *a)* by noting the color changes of natural dyes extracted from cellular tissues and comparing them with the color of living cell, *b)* by exposing cells to the solutions of indicators either by immersion or by injecting into the circulation (of animals), and *c)* by injecting the indicator directly into the protoplasm of cells by means of micropipettes.

In another paper on the oxidation-reduction potential of amoeba and starfish protoplasm, Chambers (1929b) obtained results which indicate that the O-R factor in the living cell is largely a function of atmospheric conditions.

Scarth (1929) observed the influence of hydrogen ion concentration on the turgor and movement of plant cells (stomatal behavior). Morning light initiates photosynthesis, resulting in a reduction of CO_2 concentration and development of a more alkaline reaction; the vacuome colloidal content quickly imbibes more water from the adjacent cells and allows more distention or turgor of guard cells to persist. Thus plant cells are adapted to regulate for a deficiency as well as for excess of CO_2.

Chambers and Kerr (1932) observed that none of the sulphonthalein indicators in aqueous solution entered healthy root hairs of *Limnobium* despite variations in the pH of the external medium. When injected directly into the vacuole they remain there and no coloration of the cytoplasm results. When injected into the cytoplasm they gradually diffuse into the vacuole until the cytoplasm becomes colorless. The reverse holds for Methyl Red. Injury to the cytoplasm results in

the passage of sulphonthalein indicators from the vacuole into the injured region. The normal cytoplasmic pH is 6.9 ± 0.2. Mechanical injury produces an acid reaction and the injured region develops a pH of 5.2 ± 0.2. The colorimetric pH of the vacuole (normally pH 5.2 ± 0.2) can be made alkaline to Chloro-phenol Red (pH > 6.2) for at least an hour without stopping cyclosis.

Gordon and Chambers (1941) made a significant contribution in determining the proper particle size of acid dyes and their diffusibility into living cells.

MISCELLANEOUS OTHER PROPERTIES

Guilliermond (1919) observed the chondriome of plant cells and noted the origin of chromoplastids and the mode of formation of xanthophyll and carotene pigments. Subsequently he (1930) made ultra-microscopic observations on plant cells, supplying a good review and illustrations of embryonic cells, nuclei, plastids, mitochondria, vacuoles, lipoid granules, etc. The cell content was divided into three kinds of plasm: *a)* protoplasme or living material (nucleoplasme, cytoplasme, chondriome), *b)* metaplasme (membrane), and *c)* paraplasme (vacuoles, globular lipoides, grains d'amidon, etc.).

Howland and Bernstein (1931) were able by an ingenious micrurgical device to determine the approximate oxygen consumption of a single-celled organism. This was accomplished by placing a single cell in a fine capillary tube adjacent to a bubble of air. KOH was drawn in on the opposite side of the air and both ends sealed with mineral oil. The decrease in volume of the gas, representing the oxygen consumed, was followed through the microscope quantitatively.

Arens (1939) determined the turgor pressure in single cells of *Nitella translucens* with a manometer.

Policard (1939) developed a method for microincineration of plant tissues. Hofmeister (1940a) made micrurgical studies on diatoms. Also Hofmeister (1940b) studied microinjection of plant cells.

French and Anson (1941) observed oxygen production by isolated chloroplasts of spinach.

Guttenberg (1943) cultivated isolated plant cells. Glaser (1943) developed techniques for germ-free culture of certain invertebrates, many of which live on microscopic plants. Earlier Glaser and Coria (1935) studied cultures and reactions of purified protozoa that normally have bacteria and yeasts in their diet.

CHAMBERS, DEAN OF MICRURGISTS

This discussion on the normal plant cell is brought to a close simply by mentioning the monumental work of Robert Chambers in this area of biological science. He initiated his micrurgical work on microscopic animal life at the Marine Biological Laboratory at Woods Hole, Massachusetts, and in the employ of medical schools at Cincinnati University (1911-1915) at Cornell University (1915-1928) and at New York University (1928-1950). From the start his interest also included the plant kingdom, and his laboratory made many significant contributions. Witness his interpretative articles following the invention of his micromanipulator: *a)* Section V in Cowdry's textbook which discusses the physical structure of animal and plant protoplasm as determined by microdissection and injection (Chambers, 1924); *b)* the Mayo Foundation Lecture which considers both animals and plants in the discussion on the physical properties of protoplasm (Chambers, 1927); *c)* the Harvey Lectures which discuss the basic nature of the living cell, first made understandable by the direct micrurgical approach (Chambers, 1928); *d)* in a Symposium at New York University on "The Body as a Biochemical Mechanism" (Chambers, 1930); and *e)* "The Living Cell" in Chapter I of Harrow and Sherwin's Textbook of Biochemistry (Chambers, 1935). His last and greatest endeavor was on "The Cell, per se", which, unfortunately, was not completed at the time of his passing (Duryea, 1957).

MICRURGY AND PHYTOPATHOLOGY

Relatively little has ever been done in the application of micrurgical methods to problems in phytopathology. The dearth of published material in this area was evident in 1939 at a Symposium on Micrurgical and Germ-Free Methods when a brief report, entitled "Micrurgy and Botany with Special Reference to Phytopathology" (Hildebrand, 1943), was given.

In Phytopathology and its sister science, bacteriology, the most important use of micrurgy has been for securing pure cultures by single-cell isolation. Wright and McCoy (1927) invented the "extension arms" accessory to the Chambers' apparatus for facilitating isolation of single bacterial cells, thus encouraging micrurgy at the University of Wisconsin. Gee and Hunt (1928) used the pipette method of single-cell technic as a routine laboratory procedure at Yale University.

Wright (1929) invented a convenient case for sterilizing and storing micropipettes. Wright, Hendrickson and Riker (1930) demonstrated the feasibility of separating mixed cultures of the hairy-root and crown-gall organisms by single-cell isolation. Riker et al (1930) employed single-cell progeny cultures as an aid in describing a new bacterial species. *(Agrobacterium rhizogenes).* Tilford (1936) employed cultures purified by the single-cell-progeny technique in describing *Corynebacterium fascians,* the cause of sweetpea and chrysanthemum fasciation. Micrurgical methods were used when describing the causal agent of cane gall of brambles (Hildebrand, 1940). Sheffield (1931, 1933, 1934) opened the door for micrurgical study of plant viruses by an examination of the formation and nature of intracellular inclusions.

The writer, developed a modified Barber's isolation procedure for certain difficult-to-isolate cell-stimulating phytopathogens. The original procedure was put into practice by Wright, Hendrickson and Riker (1930).

Perhaps the most classical contribution in bacterial cytopathology to date is the outstanding micrurgical work of Banfield on bacterial cancer (or crown gall) in plants in the early 1930's while he was on a National Research Council Fellowship at the University of Chicago.

BACTERIAL INVESTIGATIONS

Banfield (1935), employing a Chambers micromanipulator and the technique of Wright and McCoy (1927), isolated the rod-shaped bodies of bacterial size from living cells of cane gall tissue only to find that they would not grow on culture media. A similar isolation study of rod-shaped bodies in crown gall tissue also gave negative results for growth. It was further noted that, when transferred to culture fluids, the cytoplasmic rods from the cytoplasm of gall cells looked different from what they did within the cells. When attempts were made to isolate and culture the masses of bacterial rods from pockets in the gall tissue, a small percentage yielded cane-gall bacteria that habitually had a perfectly regular clear-cut outline and a homogeneous opalescent interior without visible structure. The rods taken from the cytoplasm of living gall cells, when isolated in a liquid medium, had a pronounced granular structure and were neither as regular nor as clear-cut in outline as bacterial rods. Further evidence to support this conclusion was gained by the use of stains, the cyto-

plasmic bodies destaining readily and the bacteria retaining the dye.

On the basis of his studies, Banfield (1935) concluded that the bacterium-like bodies, observed by Smith et al (1912) and later by Pinoy (1925) and Nemec (1928) within tissue cells of crown galls were normal elements of the chondriome of the cells and not bacteria, as at one time believed, for the following reasons: *a)* the plastids of most seed plants at certain stages in cell maturation are similar to if not identical with phytopathogenic bacteria in size and form; also the tendency of plastids in the cells of seed plants to aggregate in bacterium-like groups within the cytoplasm is general; *b)* equivalence in size, form and staining reaction of bacteria and plastids at certain stages of cellular maturation in cane gall and crown gall tissues on the black raspberry has been demonstrated; *c)* plastids commonly are more readily fixed and have a greater affinity for stains than mitochondria; selective staining of plastids was readily attained after fixation in fluids containing acetic acid, such as formal-acetic-alcohol, used by Smith et al (1912); which property was found true of plastids in cane gall and crown gall tissue in fluids of Nemec (1928); *d)* bacteria were very limited in number in crown gall tissue of black raspberry; hence the probability of not observing them and of mistaking selectively stained elements of the chondrione for the bacteria is considerable; *e)* it was demonstrated in this research that cane gall bacteria are primarily intercellular and are not found in living cells of gall tissue on black raspberry, which applies also to the tissues of crown galls induced on black raspberry.

In pathic cells the condition induced by the cane gall organism assumes states and arrangements of its contained bodies—mitochondria, plastids, and fat—such as granular, alveolar, reticular or 3-dimensioned network patterns. The visible structure of healthy cells is that of a polyphasic complex composed of a clear ground substance without visible structure, in which the spherical, rod-like or thread-like mitochondria and spherical fat globules are dispersed and in which larger starch granulas or plastids may also be dispersed or aggregated.

Similar studies, many of which were not published, were conducted by the writer (Hildebrand, 1943). Rod-shaped bodies, simulating bacteria in size, have been isolated from cells near the periphery of young cane galls and crown galls, but in no case was growth obtained. Bacteria injected into thin slices of gall tissue mounted on a coverslip were reisolated from the intercellular space, but thus far attempts to

locate pathogenic bacteria within living host cells have all been unsuccessful. It suffices here to state that non-living intracellular bodies, simulating the morphology of bacteria, were observed only in old senile cells.

SINGLE-CELL ISOLATION

Culture purification by single-cell isolation has always been the standard practice in my laboratory. Single-cell isolation was used most extensively in studies on strains of the fire-blight organism (Hildebrand, 1940). The technique employed (Hildebrand, 1938) involved isolation during 1932 of approximately 1000 viable cells from about 100 parent cultures assembled from wherever the disease was known around the world.

One phase was a study of comparative behavior of 136 cultures over a five-year period. When transferred biweekly and subjected to numerous physiological, morphological and pathogenetic tests, isolates or strains showed numerous small (phenotypic) differences, but no strain displayed significant disease resistance in the pear-breeding program (Hildebrand, 1954).

In a study of growth rates or generation times of phytopathogenic bacteria (Hildebrand, 1938b), parent and single-spore progeny cultures were compared.

When two fungi used by students in their thesis problems could not be purified from bacterial contamination by any other means, my micrurgical technique proved rapid and certain, and only about an hour was required to obtain ten single-spore progeny from each parent culture. The contaminants were removed from the slimy spore surface by washing each spore in a physiological saline solution to cut the slime and free it from the contaminants.

SINGLE-CELL INOCULATION

Excised unopened clusters of apple blossoms provided the ideal subject for determining the infectivity of the fire-blight organism (Hildebrand, 1937). Handled under aseptic conditions in a moist chamber, the nectary secreted germ-free nectar which proved to be an ideal growth medium for this organism. The host-parasite relationship was established by inoculation of single bacterial cells into the nectar secretion of each nectary. To ready the floral nectary for inoculation, the writer placed excised unopened blossoms in a moist

chamber overnight or long enough for the hygroscopic nectar (with a secretion concentration of about 20 percent sugar) to be diluted to five percent sugar or less before inoculation, by which time the blossoms were open and the nectar was filling the nectary. About 24 hours after inoculation single bacterial cells had multiplied into the hundreds of thousands and imparted to the nectar a milky turbidity. The mechanism of infection was through the recently discovered nectar-secreting stomates (Hildebrand and MacDaniels, 1935) distributed over the inner lining of the nectarial surface. In pathological histology it was demonstrated that a toxin was secreted into the intercellular habitat by the bacteria. Thus a toxic plasmolysis set in within two days, causing rapid death or blighting of the blossoms (blossom blight) and stem tissues (cankers).

SINGLE-CELL VS. MULTICELLULAR INOCULATIONS WITH THE CROWN-GALL ORGANISM

The infection mechanism with the crown gall organism in tomato was studied micrurgically (Hildebrand, 1941). To begin with, the single bacterial cells grew as readily in sterile juice extract from young tomato plants as in yeast-extract broth, the viability percentage being over 90. When introduced into deep wounds on tomato stems, single bacterial cells produced crown galls in ten to 60 percent of the trials compared with ten percent or less for shallow wounds. Similarly when ten or more bacterial cells were used, gall formation resulted in about 90 percent of the trials with deep wounds compared with 20 percent or less of the trials with shallow wounds. These studies suggest that the kind of wound is more important that the number of bacteria for initiating infection by the crown gall organism in tomato.

Similar studies were mentioned in another report (Hildebrand, 1943). Both the depth of the shallow wound and the number of bacteria introduced into the infection court seem to govern the size of resulting galls. Gall development at the end of one month following inoculation of a single bacterial cell and many cells into shallow wounds was compared. In this one instance the gall resulting from single-cell inoculation was very much smaller. When introduced into deep wounds, the single cells, which gave positive results, always produced large galls. The smaller gall can be attributed to the shallower penetration by the single cell compared with penetration of many bacteria.

The last series in this micrurgical study of crown-gall infection in

tomato (Hildebrand, 1942) extended and supported the previous work. A single cell in deep wounds was adequate for inducing gall formation. Single bacteria grew readily in the juice extract in microculture. The sap in the wound cavity liberated from cells in the wounding operation also supported growth of the organism. Isolations from wounds about five days after inoculation and before symptoms appeared demonstrated large increases in numbers of bacteria in the wound sap.

Four series of inoculations were made with the crown-gall organism into 42, 38, 16 and 15 hair cells, all with negative results for gall formation. Further studies demonstrated that when crown gall bacteria were injected into the living cells of tomato stems, they never induced gall formation or any other recognizable disturbance in the inoculated cells or the neighboring tissues. Also the bacteria failed to survive inside the tomato cells, indicating that the cell interior is an unfavorable medium for survival. The fact that living bacteria were recovered in only two instances from many inoculated cells after about three weeks incubation strongly supports Banfield's work and contention (Banfield, 1935) that cell-stimulating bacteria operate only from the intercellular spaces between cells.

A very minute injury visible only through a microscope because only three to five epidermal cells seem to be involved is large enough for gall formation. Tiny wounds were produced by gently rubbing a polished steel needle, coated with a film of bacterial suspension, on young tomato stems and petioles so as to gently crush hair cells. Apparently the sub-epidermal cells at the wound site were stimulated to produce tiny galls which varied in size according to the extent of injury. From the tiniest galls no bacteria were recovered by the poured-plate technique. Injection of large numbers of crown gall bacteria into epidermal cells never caused gall production.

Histological studies, conducted jointly with A. C. Braun at the Rockefeller Institute in 1939-1940, revealed the presence of few bacteria in tiny galls of tomato. These were so few as to make their exact location difficult to establish. Consequently this phase of the work remains incomplete indefinitely or until a better micrurgical approach is perfected.

VIRUS INVESTIGATIONS

Prior to the Symposium on Micrurgical and Germ-Free Methods at Notre Dame University in November, 1939, the writer studied the

mouth parts of aphids, the mechanical transmitters of plant viruses. This was done in an endeavor to design "a mechanical insect" to inject into and withdraw from living plant tissues or cells minute amounts of inoculum or other material. Such a precision device still is not perfected. However, micropipettes controlled by the Chambers' Micromanipulator and of approximately the same dimensions as aphid stylets were used to inoculate virus extract of aucuba mosaic into the epidermal cells of tomato with success, thus supplying a clue to the mechanism of virus inoculation in plants by or through perforation wounding.

In his investigation on the mechanism of transmission of plant viruses by insect vectors, Storey (1933) employed semi-micrurgical methods for introducing the virus into the insects. A fine needle or pipette containing the virus extract was held in the hand while the operation was performed under a dissecting microscope. The method of inoculation of *Cicadulina mbila,* the insect vector of the streak disease of maize, involved steadying the insect with a "holding needle" in the left hand and puncturing the abdomen with the "inoculating needle" in the right hand. Another method, that of "deep inoculation", involved thrusting the point of the needle through the anterior part of the insect's abdomen. For heavy inoculation a special "reservoir needle" was used. The point of the needle having been introduced into the abdomen of the insect, the fluid was drawn down from the reservoir to the puncture with the point of the holding needle, and it was possible to see that a unmeasurable quantity of the fluid passed into the puncture. Storey also injected virus extracts into the bodies (abdomens or thighs) of the leafhoppers by means of a micropipette sealed to the end of a so-called micrometer syringe.

Employing micrurgical technique, Fife and Frampton (1936) supplied a plausible explanation for the method by which the leafhopper vector of the curly top virus of sugar beet is able to locate and select the phloem tissue in which to feed. This explanation was based on the existence of a pH gradient between the phloem and the parenchyma. By means of a quinhydrone microelectrode for pH measurements, it was demonstrated that the phloem was more alkaline than the surrounding parenchyma, resulting in a pH gradient. The fact that the saliva of the leafhopper was alkaline like the phloem led to the hypothesis that the pH gradient was used by the insect in locating the phloem, there being presumably an undesirable reaction to the

insect setae or feeding mechanism until the alkaline tissue was penetrated.

INTRACELLULAR INCLUSIONS

Cytoplasmic inclusions are formed in plants affected with various virus diseases (tobacco mosaic, aucuba mosaic, Hyoscyamus III), although they are never found in plants infected with cucumber mosaic virus. Inclusions are never formed in the sap of vacuoles of infected cells, probably because they do not contain virus. Plastids are supposed to be related to mitochondria. The possible origin of viruses from mitochondria has been suggested, but this idea is as yet only speculative. Cells infected with a plant virus may or may not show pathological changes, depending on the virus and on the cell function (Esau, 1938). There is a diminution in number and size of chloroplasts in plants affected with "yellows" diseases, but it is uncertain that there is an attending decrease in the yellow pigments (carotene, xanthophyll). The most direct morphological manifestation of virus infection in a host cell is the production of intracellular inclusions.

Sheffield (1931) was the first to make a detailed study on the formation of intracellular inclusions in virus-infected plants. In plants affected with aucuba mosaic, inclusions are produced by the gradual aggregation and fusion of minute particles which circulate in the cytoplasmic stream within a few days after inoculation. It appears that these inclusions are formed by the susceptible host cell in response to stimulation by the virus. This raises the question whether the virus is confined to inclusions or is also dispersed throughout the cytoplasm. A description is given of the mode of formation of intracellular inclusions induced by aucuba mosaic of tomato in *Solanum nigrum, S. nodiflorum, Lycopersicon esculentum, Nicotiana tabacum* and *Hyoscyamus niger.*

Soon after infection the rate of cytoplasmic streaming is increased, the minute particles of protein appearing in the cytoplasm being carried passively around the cell. These aggregates fuse and form large masses still carried passively but more slowly around the cell. Fusion continues until all the protein material is contained in one granular mass or occasionally more. In the two *Solanum* species this mass becomes rounded; it may lose its granular appearance and become vacuolated. In *N. tabacum* and *H. niger* the body does not always round off but remains an irregular granular mass which may become vacuolated. After the spherical body is formed, a spike-like crystal appears in the

cell which remains at rest for several weeks. Ultimately the body breaks down, giving a number of protein crystals. After several months these dissolve.

In *H. niger* the inclusion bodies are confined to the chlorotic areas, where they are abundant in all tissues. In other species they are distributed throughout the green and yellow areas of tissue. They are abundant in hairs, less abundant in the epidermis, and very rare in palisade and spongy tissues.

Sheffield (1933) noted with aucuba mosaic of tomato that intracellular inclusions are not found in meristematic tissue but that incipient bodies appear when the cells are increasing in size and after plastid development is well advanced. For this reason inclusion bodies are formed indiscriminately in green and chlorotic areas, the virus presumably having reached the green tissues too late to inhibit plastid development. Absence of chlorophyll is brought about by inhibition or destruction of plastid primordia by the virus. If not prevented or inhibited at a very early stage, perfectly normal plastids form. Mature plastids are never affected by virus, but those in intermediate stages may be.

Sheffield (1934) experimented on the nature of intracellular inclusions associated with aucuba mosaic and Hyoscyamus III viruses, compared with those associated with tobacco mosaic virus. With the first two viruses, inclusion bodies are formed by aggregation and fusion of minute particles which appear in the cytoplasmic stream. In plants affected with tobacco mosaic virus, an amoeba-like body that persists for some weeks before suddenly disappearing is produced. It is accompanied by striate material, all of which ultimately fuse into one large body. All reagents used induced stimulation of the cytoplasmic stream similar to the initial sign of virus infection. With the salts of molybdic acid, all the cytological abnormalities due to aucuba mosaic and Hyoscyamus III disease have been imitated. Treatment with lactic acid induces formation of amoeboid bodies like the X-bodies of tobacco mosaic virus, but these bodies persist only a few hours. These experiments support the view that the intracellular inclusions associated with plant virus diseases are essentially products of host cells.

MICRODISSECTION

Livingston and Duggar (1934) employed micrurgical procedures and made the first study on the location and concentration within the

host cell of the virus of common tobacco mosaic. Stem slices of young diseased tobacco plants bearing infected hairs were mounted in 20-percent gelatin on the glass cover slip placed over a moist chamber and studied under higher magnifications. By use of a Chambers' micromanipulator, fine micropipettes with openings from 0.5 to 1.0 μ in diameter were introduced into the hair cells so as to withdraw and study the contents separately for virus and various components. Experimental evidence supports the view that the virus of tobacco mosaic occurs primarily, if not solely, in the protoplasmic component, not in the vacuole of the cell. Intracellular bodies in the hairs at least were coincident with or accompanied by relatively high virus concentration. The inclusion bodies (both amoeboid and crystalline) were fragile, disintegrating on contact with the microneedle or pipette.

Using micrurgical methods, Sheffield (1936) studied the susceptibility of plant cells to viruses. Inoculations by micropipette into single cells (hair, palisade or phloem tissue) yielded only about one-tenth of the expected number of infections. This study employed three viruses—tobacco mosaic, aucuba mosaic and Hyoscyamus III. The apparatus included a Janse and Peterfi micromanipulator bearing one microscope and a second microscope placed by the micromanipulator in such a position that the tip of the pipette could be swung around from the optical axis of one microscope to that of the other. Although intracellular inclusions may occur in every cell over large areas of the epidermis, they were not found in the guard cells of the stomata, possibly because no protoplasmic connections could be shown to exist between guard cells and the surrounding tissues (cells). Support is lent to the view that when virus moves in the ground tissues of the host, it is carried from cell to cell along the protoplasmic bridges.

Several phases of the work by Sheffield were repeated and extended by the writer (Hildebrand, 1943, 1958) with emphasis on the mechanism of inoculation and usually with but one virus (aucuba mosaic) and one host (tomato). The micropipette injection of intact single hair and epidermal cells with minute amounts of fresh virus extract gave positive but variable results ranging from 18 to 52 percent. The mechanism of plant virus inoculation (Hildebrand, 1943, 1958), based on work conducted between 1937 and 1939, was described as a pulsing of protoplasm through the wound perforation by which presumably all viruses gain entry, a universal phenomenon in plant cells.

Sheffield (1939a) pointed out the difficulties attending micrurgical

studies on virus-infected plants. In her study she used principally excised cutinized hair cells of *Solanum nodiflorum* infected with aucuba mosaic. Both by contact with a microneedle and with slight mechanical pressure on the outside of the cell, the amorphous inclusion bodies of aucuba mosaic therein were destroyed immediately without injuring the cell. However, despite their fragility, these inclusions can be removed intact from the cells if mechanical pressure is avoided in the region of the inclusion and a suitable mounting fluid is used.

Sheffield (1939a) also perfected a method for studying the electrokinetic properties of plant cells. The reaction of the cell contents of *S. nodiflorum* and *H. niger* in both healthy and diseased plants was the same, pH 6.8. For making these determinations a dry crystal of dye was introduced into a cell by needle puncture. The contents of the epidermis and palisade tissue were always less acidic than that of the hair cells, the pH being between 6.8 and 7.2. No differences were observed in reaction in any type of healthy and diseased tissue studied.

The virus content of individual hair cells were determined by collecting inclusions from several hairs for each test (with the number, in multiples of 10, based on volume calculations) and inoculating these into leaves which would show local lesions. Purified virus was used as a standard for comparison. The technique was to select a cell next to the basal one of a hair with an inclusion near its base and to arrange it in a hanging drop of 0.1 M potassium phthalate solution buffered to pH 3.4. The hair was held at the base by a large needle or forceps so that it was flat against the coverglass. The hair was then divided into two pieces by a cut just below the top wall of the selected cell. The inclusion was removed by exerting a very gentle pressure at the base of the cell or by replacing the cutting needle by a pipette and removing the body from the cell by suction. The needles were replaced by pipettes (one containing phthalate buffer solution). The body was deposited on glass and washing solution added by one pipette and removed by the other several times. Then it was picked up by the former and dropped into a tube containing a small quantity of water and stored in a refrigerator until 10 to 20 inclusions were collected, which required several days. It was found in these tests that the inclusions contained more virus than the cytoplasm.

Every method of determining pH of plant cell content is open to grave criticism since it involves wounding, which of itself increases hydrogen ion concentration, and a time lapse between wounding and

reading. To combat this criticism Sheffield adopted Peterfi's suggestion for improvement, namely, use of a little dry dye on a spot of vaseline from which it is picked up on the tip of a microneedle used to puncture the cell. The indicator dissolves at once in the cell sap and the color appears immediately.

Sheffield (1939b) summarizes the effects of plant virus diseases on their hosts. The first effect seems invariably to be stimulation. Streaming of protoplasm is greatly accelerated. In rare cases mitosis may be induced even in adult cells. The stimulating effect, which passes in a few days, is usually followed by retardation of growth. A few days later histological and cytological changes bring about a variety of symptoms. Some virus diseases cause no apparent abnormalities in the cells of a host plant, but others induce production of inclusion bodies. In some cases these are amorphous and in others crystalline. The different types of intracellular inclusions are discussed and explained from excellent illustrations.

MICROINJECTION AND MISCELLANY

Storey (1938) proposed an "independence hypothesis" in which "insufficient doses" of virus, delivered by numerous insects at different wound points on one or more leaves of the same plant, can never combine to give a "sufficient dose" for infection. Others, including the writer, have used the term "minimum infective dose" for the same phenomenon. Work on rapid-transmission techniques with the stone-fruit viruses (Hildebrand, 1941, 1942a, 1942b) demonstrated almost from the start that only one bud or one scion is needed to effect transmission of any of the numerous viruses studied. Unpublished work on sweetpotato internal cork has repeatedly shown that the reason several aphids are better than a single aphid is that only certain individuals carry the minimum infective dose and that others are non-infective or innocuous.

Storey (1939) made a thorough critical study of the mechanism of the transmission of plant viruses by insect vectors. His work on the leafhopper's mouth parts and saliva demonstrated how the feeding (perforation, inoculation) function is performed with the stylets carrying the viruliferous saliva into the plants. This phenomenon appears to be universal for the leafhopper type of vector. A salivary secretion seals the stylets in the perforation position so as to carry on the feeding process through a closed system.

Doolittle and Walker (1928) studied the aphid vector of cucumber mosaic virus and demonstrated that this virus can be transmitted as a contaminant of the stylets of aphids. This view has since come to be widely accepted as explaining the mechanistic or accidental nature of aphid transmission generally. Thus in physical relation to the aphid's stylets the virus would be non-persistent because of being carried as a surface contaminant that would wear or rub off.

Benda (1956a) studied infection following virus inoculation of single plant cells and raised problems by his query: "If 1 lesion is due to 1 virus particle", then, *a)* Why can only a small fraction ($<$ 1 in billion) of cells exposed be infected? *b)* How is the manner or mode of lesion growth about the cell initially infected to be described? *c)* can two strains of a virus be introduced into a single cell to give a local lesion containing two strains? *d)* Is there a technique whereby a marked cell on a leaf may be inoculated with virus?

The same year Benda (1956b) reported the results of infection of *Nicotiana glutinosa* following microinjection of two strains of tobacco mosaic virus into a single cell. When a mixture of the ordinary strain and the yellow aucuba strain of tobacco mosaic virus was injected into a single hair cell of an excised seedling leaf, either or both strains were subsequently detected in the lesion. The ordinary strain alone was found in 14 of the 20 lesions examined, the yellow aucuba strain alone in one lesion, and both strains in five lesions. With a single exception, lesions appeared only on leaves in which the inoculated hair remained alive at least one day. Of 1157 hairs inoculated, 694 remained alive one day, and 20 of these subsequently developed lesions; in contrast, only one lesion developed among 463 which collapsed within one day. The inoculated hair was approximately at the center of the lesion that developed on the second day. The inoculated hair usually collapsed on the third day, but an occasional hair was still alive the fourth day.

Beale (1937) repeated Stanley's (1936) procedure for isolating crystalline tobacco-virus protein from five sources. By addition of dilute acid or salt to water extract of diseased tissue, the crystalline plates were transformed to needle crystals in all hosts tested. The reagents used were H_2SO_4, HCl, acetic acid, nitric acid and saturated magnesium sulfate solution. Intracellular crystalline plates observed may be more complex in chemical composition than Stanley's crystals. Beale concluded that intracellular crystalline deposits are sources of

Stanley's crystalline tobacco-virus protein for the following reasons: *a)* the two crystalline groups were present in large amounts; *b)* there was a striking similarity in gross appearance of the needles precipitated in the cell and isolated from virus extract; *c)* the acidity and alkalinity at which Stanley reports denaturation of the protein correspond closely to the reactions at either end of the pH range at which the intracellular crystals go into solution and are not subsequently recrystallizable. Thus it is concluded that concentration is an important factor in the intracellular crystallization of tobacco virus protein.

Steere and Williams (1953) identified the crystalline inclusion bodies extracted intact from plant cells infected with tobacco mosaic virus. They stated: "A process is described for the intact removal of the crystalline inclusion bodies from hair cells of tobacco plants infected with tobacco mosaic virus. The method is a simple one of freeze-drying, followed by microdissection of the crystals from the dry hair cell. By electron microscopy it is found that crystals consist apparently of nothing but particles of tobacco mosaic virus and a volatile solvent. The total dry volume of the particles accounts for approximately one-third the volume of the crystal. It has been demonstrated that the main infective virus particles are present among those comprising the crystalline inclusion bodies".

McWhorter and Price (1949) demonstrated that tobacco plants simultaneously injected with tobacco mosaic and mild etch viruses show within the same cell the intracytoplasmic crystalline plates of the mosaic and the intranuclear plates of the etch viruses.

Maramorosch (1956) successfully used semi-automatic equipment for injecting insects with measured amounts of liquids containing viruses or toxic substances. Using Storey's (1938) puncture technique, Maramorosch et al. (1949) made successful mechanical transmissions of Black's plant tumor virus to an insect vector.

Bawden and Kassanis (1950) found no evidence that host nutrition had any consistent effect on the intrinsic infectivity of the causal virus. They proved that the amount of tobacco mosaic virus in systemically infected tobacco plants varied greatly with the mineral nutrition of the plants and was related to the effects on plant growth. Host nutrition by altering plant size indirectly altered virus production so that poor nutrition produced a low yield of virus, and vice versa.

Hildebrand (1956) found that the internal cork virus of sweet-potato induced distinctive leaf spots on the Triumph variety. In the

young translucent spots the virus apparently performed an enzyme function by separating the parenchyma cells from each other so as to float freely apart. The separated palisade and spongy parenchyma cells, with their complement of chloroplastids, were ideal material for micrurgical studies.

ELECTRON MICROSCOPY

The electron microscope brings us closer to an understanding of virus origin, which is of a dual nature: *a)* the relation of viruses to cell components and *b)* the origin of cell components (Luria, 1953). Each virus has its gene complement, its genome, and just as obviously must possess the elementary prime factor component, the "spark of life" gene.

The electron microscope provides some evidence on the formation of virus inclusions. Virus-infected cells from animal tissue cultures were used first for electron microscopy (Porter et al, 1945). Wyckoff (1949) reviewed this subject. The introduction of perfected sectioning techniques has made it possible to use extremely thin slices for direct electron microscopy of several virus inclusions, but many viruses neither form intracellular inclusions nor produce distinctive macromolecular bodies or crystals.

Observations on the formation of intracellular inclusions generally suggest that reproduction of virus takes place by a process in which mature virus particles are not recognizable. In other words, the final stage of particle formation of some viruses can be an aspect of the intracellular life cycle which is not attained by many of the younger more labile viruses. Consequently the non-particulate homogeneous stage of viroplasm which contains the elementary building blocks will produce infection without differentiation into distinctive particles.

Intranuclear inclusions were reported (Luria, 1953) in a number of virus infections. These inclusions were more acidophilic than the nuclear material, and no observations were made of mitosis ever occurring in cells with intranuclear inclusions. Virus was demonstrated in herpes inclusions isolated by micromanipulation (Baumgartner, 1935). A particular group of intranuclear inclusions are the "polyhedra" found in a number of infectious diseases of insect larvae (Bergold, 1953; Trager, 1935). These crystalline inclusions contain the virus in the form of rod-shape particles, their presumptive precursors and a native protein serologically related to the virus.

FUNGUS INVESTIGATIONS

Micrurgical methods widely used for single-spore isolation (Hildebrand, 1938, 1950) also offer promise in studies on the cytopathology of fungi, but this subject has been largely neglected.

In a previous report (Hildebrand, 1943) a study of the mechanism of cell-wall penetration within apple fruits by *Phytophthora cactorum*, a rot-producing organism, was cited. Thin slices of fruit tissue were mounted in a moist chamber and a small inoculum of fungus tissue placed nearby. It was noted that the hyphal tips, upon reaching the wall of an apple cell, became firmly attached and after being at rest for about five minutes passed through a constriction in the wall, moved rapidly to the next wall, and repeated the performance across the microscopic field. With a microrod controlled by a micromanipulator, the hyphal tips were difficult to dislodge after they had been at rest against the wall for about three minutes. Pressing a fabricated microrod approximately the size and shape of the hyphal tips against the cell wall failed to perforate it or pass through, instead it caused the wall to fold up like a tough membrane. However, if the hyphal tips, after being at rest approximately three minutes, were dislodged, the microrod could be easily pushed through the wall at the contact point, suggesting that the hyphal tips secrete a substance which softens the wall preceding penetration. That secretion occurred only at the tip and that pressure, in addition to the softening action, was also operating was indicated by the fact that when the hyphal tips came to rest at angles less than about 50 degrees, the tips were unable to penetrate and instead followed along the wall until the angle was satisfactory.

One unpublished micrurgical study consisted of isolation in lineal order of the eight ascospores from about 25 asci of the brown rot fungus *(Monilinia fruticola)* as the apothecia (produced on peach mummies) were nearing maturity. In nearly all instances five or more spores from each ascus germinated. In three instances, as many as seven ascospores of the eight germinated in culture, but in no instance did all eight germinate and grow.

In another micrurgical study single and multiple ascospores and conidia were used to inoculate peach blossoms, but they produced infection only when placed singly or in groups in tiny wounds. Single conidia germinated readily in juice extract from mature fruit. By the very nature of spore dispersion and dissemination in nature, it is

probably a truism that the majority of infections by fungi in the Ascomycetes and Fungi Imperfecti are initiated by individual spores.

At the Plant Breeding Station in Elvas, Portugal, Santiago (1956) stressed the importance of single-spore technique in studies on the cereal rusts. When large numbers of biotypes were found within each physiological race, he concluded that race differentiation was more satisfactorily obtained by single spore culture than by single pustule culture. He contends that the single pustule may not actually represent a clonal line. Therefore he recommends regular use of single-spore culture for breeding rust-resistant wheat.

In breeding plants for resistance to fungus pathogens it is important to evaluate the inoculum at yearly intervals for virulence. The stem rot, or wilt, of sweetpotato is a case at point because here breeding for disease resistance is an important phase of crop improvement, and this calls for maintenance and renewal by micrurgical means of virulent cultures of the causal species of *Fusarium* (Hildebrand et al., 1958).

The importance of single-spore technique in investigations on disease resistance to fungi should not be underestimated.

DISCUSSION

This review is largely concerned with and limited to selected cases from but one phase of the new science of micrurgy: the Plant World. Battista (1947) considers the science of micrurgy in full breadth and scope, and thus covers the use of midget tools under a microscope to delve into all the fascinating worlds of the infinitely small. He points out that it is now possible to tamper with chromosomes within individual living cells, pull out and tear apart rust particles on a metal surface, or perform a complete chemical analysis on a pin point of blood. Possibilities are equally fascinating in medical, biological and chemical fields—complete blood analysis, pure strains of yeast isolated and grown in pure culture medium and one thoroughbred strain promises a better textured loaf of bread. Surgical feats are performed on tissues of man, animals and plants and on individual living cells; nuclei of individual amoeba can be removed and studied. These studies are legion.

Battista relates how micrurgy was linked to the difficult desert fighting in North Africa when Tobruk and El Alamein headlined the war news. He heard Dr. George F. Head describe some of the work which he and his associates had done in the micrurgical laboratories

of the Fairchild Engine and Airplane Corporation. When the fighting in North Africa was bogged down by engine trouble, they examined the rust-eaten metal surface under very high magnification. Employing tiny chisels (25 microns or 1/1000 inch), they hacked out infinitesimal nicks of the deeply imbedded sand and rust. Then grasping the rust particles with midget tweezers, they tore them apart with minute hammers and magnets, and analyzed their static charge and chemical nature. This information pointed the way to the manufacture of airplane engines and parts which stood up under the desert conditions of warfare.

Micrurgists also study the surface of metals for defects. One mirco-specialist assembled a micromagnet for removing minute particles of iron from crevices on the surface of a non-magnetic metal. In another instance, tiny steel scrapers were used to remove molecular layers of oxide films for microchemical analyses. The smallest electronic tubes known to date were assembled with the aid of micromanipulators. Also the most recent information on electroplating of elements was obtained with tiny microelectrolysis cells and microtools under the microscope.

Battista relates how the biologist pioneer of micrurgical techniques, Robert Chambers, told him: "The science of micrurgy will prove to be a boon to investigators of the microscopic worlds. The new techniques may prove as important to the microspecialist as a pole reaching to the surface of the moon could be to the astronomer".

Battista predicts that "as the value of working with tiny tools under the microscope becomes more universally known and applied, micrurgy will undoubtedly achieve status as a major science . . . No one can yet chart the invisible worlds micrurgy will conquer". My only knowledge of the application of micrurgy to the physical world came in the early thirties when, as a micrurgist specialist, I was invited to a micrurgical laboratory in Rochester, New York, to observe chemical analysis of minute particles with a microforge.

LITERATURE CITED

1. ALLEN, C. E. The potentialities of a cell. Amer. Jour. Bot. **10**: 387-398. 1923.
2. ARENS, K. Determination of turgor pressure in a single cell with manometer. Planta **30**: 113-117. 1939.
3. BANFIELD, W. M. Studies in cellular pathology. I. Effects of cane gall bacteria upon gall tissue cells of the black raspberry. Bot. Gaz. **97**: 193-239. 1935.

4. BARBER, M. A. A new method of isolating micro-organisms. Jour. Kan. Med. Soc. **4**: 487-494. 1904.
5. ———— The rate of multiplication of *Bacillus coli* at different temperatures. Jour. Inf. Dis. **5**: 380-383. 1908.
6. ———— A technic for the inoculation of bacteria and other substances into living cells. Jour. Inf. Dis. **8**: 348-360. 1911a.
7. ———— The effect on the protoplasm of *Nitella* of various chemical substances and of microorganisms introduced into the cavity of the living cell. Jour. Inf. Dis. **9**: 117-129. 1911b.
8. ———— The pipette method in the isolation of single microorganisms and in the inoculation of substances into living cells. Philippine Jour. Sci. B. Trop. Med. **9** (4): 307-360. 1914.
9. ———— Use of the single cell method in obtaining pure cultures of anaerobes. Jour. Exp. Med. **32**: 295-312. 1920.
10. BATTISTA, O. A. Micrurgy—a new science. Columbia Mag. **26**: 4, 23. 1947.
11. BAUMGARTNER, G. Infektionsversuche mit isolierten oxychromatischen Einschlussen des Herpes. Schweiz. Med. Wochenschr. **16**: 759-760. 1935.
12. BAWDEN, F. C., and B. KASSANIS. Some effects of host plant nutrition on the multiplication of viruses. Ann. Appl. Biol. **37**: 215-227. 1950.
13. BAYLISS, W. M. The properties of colloidal systems. IV. Reversible gellation in living protoplasm. Proc. Roy. Soc. B. **91**: 196-201. 1920.
14. BEALE, H. P. Relation of Stanley's crystalline tobacco-virus protein to intracellular crystalline deposits. Contr. Boyce Thomp. Inst. **8**: 413-431. 1937.
15. BENDA, G. T. A. Infection following virus inoculation of single plant cells. [Abs.] Plant Physiol. **31** (suppl.): X 1956a.
16. ———— Infection of *Nicotiana glutinosa* L. following injection of two strains of tobacco mosaic virus into a single cell. Virology **2**: 820-827. 1956b.
17. BERGOLD, G. H. Insect viruses. Adv. Virus Res. **1**: 91-139. 1953.
18. BERKELEY, G. H. Reactions of plants to virus infection. 34th Rep. Quebec Soc. Protect. Plants: 101-115. 1952.
19. BULLER, A. H. R. Researches on Fungi. Vol. IV. Further observations on the Coprini together with some investigations on social organization and sex in the Hymenomycetes. Longmans, Green & Co. London. New York. 1931.
20. ———— Researches on Fungi. Vol. V. Hyphal fusions and protoplasmic streaming in the higher fungi, together with an account of the production and liberation of spores in *Sporobolomyces*, *Tilletia*, and *Sphaerobolus*. Longmans, Green & Co. London. New York. 1933.
21. BURCH, G. E. A simple method for accurate injection of small volumes of fluid. Proc. Soc. Exp. Biol. & Med. **40**: 676-678. 1939.
22. BÜTSCHLI, O. (1) Studien über die ersten Entwickelungsvorgange der Eizelle, die Zelltheilung und die Konjugation der Infusorien. Abhandl der Senckenberg. naturforsch. Ges. 10. 1876. (2) Über die Entstehung des Schwarmsprosslings der *Podophaya quadripartita* Clp. u Schm. Legendi f. die Zoologischen Wiss. Polytech. Zur Carlsruhe: 1-23. 1876.
23. CASTLE, E. S. Discontinuous growth of single plant cells measured at short intervals, and the theory of intussusception. Jour. Cell. & Compar. Physiol. **15**: 285-298. 1940.
24. CHAMBERS, R. Some physical properties of cell nucleus. Science **40**: 824-827. 1914.

25. ———— Microdissection studies on the germ cell. Science **41**: 290-293. 1915a.
26. ———— Microdissection studies on the physical properties of protoplasm. Lancet-Clinic. **113** (13): 363-365. 1915b.
27. ———— The microvivisection method. Biol. Bul. **34**: 121-136. 1918.
28. ———— A simple apparatus for micro-manipulation under the highest magnifications of the microscope. Science **54**: 411-413. 1921.
29. ———— New apparatus and methods for the dissection and injection of living cells. Anat. Rec. **24**: 1-19. 1922.
30. ———— The physical structure of protoplasm as determined by microdissection and injection. Section V: 237-309. Cowdry, E. V. General Cytology. Ed. 2. Univ. Chicago Press. 754 pp. 1924.
31. ———— The physical properties of protoplasm. Mayo Foundation Lectures: 113-132. W. B. Saunders Co. Philadelphia and London. 244 pp. 1927.
32. ———— The nature of the living cell as revealed by micro-dissection. Harvey Lecture Ser. XXII: 41-58. 1928a.
33. ———— The nature of living cells as revealed by micro-manipulation. Alexander, J. Colloidal Chemistry, Theoretical and Applied. Vol. II: 467-486 The Chemical Catalog Co. New York: 1928b.
34. ———— Hydrogen ion concentration of protoplasm. Bul. Nat. Res. Council, No. 69: 37-47. Washington, D.C. 1929a.
35. ———— The oxidation-reduction potential of protoplasm. Bul. Nat. Res. Council. No. 69: 48-50. Washington, D.C. 1929b.
36. ———— The body as a biochemical mechanism: Is it? Symposium on Physical Education & Health. New York Univ. Press., 118-126, New York. 1930a.
37. ———— Vital staining with Methyl Red. Proc. Soc. Exp. Biol. & Med. **27**: 809-811. 1930b.
38. ———— The living cell. Chap. I: 17-39. Harrow, B. and C. P. Sherwin. A Textbook of Biochemistry. W. B. Saunders Co. Philadelphia. 797 pp. 1935.
39. ———— Blue nuclei in tulip petals. Amer. Jour. Bot. **28**: 445-446. 1941.
40. ———— and M. Black. Electrolytes and nuclear structure of the cells of the onion bulb epidermis. Amer. Jour. Bot. **28**: 364-371. 1941.
41. ———— and H. P. Hale. The formation of ice in protoplasm. Proc. Roy. Soc. B **110**: 336-352. 1932.
42. ———— and K. Hofler. Micrurgical studies on the tonoplast of *Allium cepa*. Protoplasma **12**: 338-355. 1931.
43. ———— and T. Kerr. Intracellular hydrion concentration studies VIII. Cytoplasm and vacuole of *Limnobium* root-hair cells. Jour. Cell & Compar. Physiol. **2**: 105-119. 1932.
44. ———— and M. J. Kopac. Micrurgical technique for the study of cellular phenomena. McClung's Handbook of Microscopical Technique: 62-109. Ed. 2. Paul B. Hoeber, Inc. New York. 698 pp. 1937.
45. ———— and H. C. Sands. A dissection of the chromosomes in the pollen mother cells of *Tradescantia virginica*. Jour. Gen. Physiol. **5**: 815-819. 1923.
46. Demoor, J. Contribution à l'étade de la physiologie de la cellule (independence fonctionnelle du protoplasme et du noyau). Arch. Biol. **13**: 163-244. 1894.
47. Doolittle, S. P., and M. N. Walker. Aphis transmission of cucumber mosaic. [Abs.] Phytopath. **18**: 143. 1928.

48. DUJARDIN, F. F. Recherches sur les organismes inferieur. Ann. Sci. Nat. II. Zool. 4: 343-376. 1835.
49. DURYEA, W. R. R. Chambers, pioneer in the study of living cells. Science 126: 645. 1957.
50. EAMES, A. J., and L. H. MACDANIELS. An Introduction to Plant Anatomy. Ed. 1 & 2. McGraw-Hill Book Co. New York. 1925, 1947.
51. EWART, A. J. On the physics and physiology of protoplasmic streaming in plants. Oxford, England. 1902.
52. FARR, W. K. X-Ray diffraction patterns of cellulose particles and interpretations of cellulose diffraction data. Contr. Boyce-Thomp. Inst. 6: 315-321. 1934.
53. ———— Certain colloidal reactions of cellulose membranes. Jour. Phys. Chem. 41: 987-995. 1937.
54 ———— The microscopic structure of plant cell membranes in relation to the micellar hypothesis. Jour. Phys. Chem. 42: 1113-1147. 1938.
55. ———— and S. H. ECKERSON. Separation of cellulose particles in membranes of cotton fibers by treatment with hydrochloric acid. Contr. Boyce-Thomp. Inst. 6: 309-313. 1934.
56. ———— and W. A. SISSON. Observations on the membranes of epidermal cells of the *Avena* coleoptile. Contr. Boyce-Thomp. Inst. 10: 127-137. 1939.
57. FIFE, J. M., and V. L. FRAMPTON. The pH gradient extending from the phloem into the parenchyma of the sugar beet and its relation to the feeding behavior of *Eutettix tenellus*. Jour. Agr. Res. 53: 581-593. 1936.
58. FLEMMING, W. Neue Beiträge zur Kenntnis der Zelle. II. Theil. Arch. Mikr. Anat. 37: 685-751. 1891.
59. FRANZ, E., E. SCHIEBOLD, and C. WEYGAND. Über den morphologischen Aufbau der Bakterienzellulose. Natarwiss. 31: 350. 1943.
60. FRENCH, C. S., and M. L. ANSON. Oxygen production by isolated chloroplasts. Amer. Jour. Bot. (Suppl.) 28: 12s. 1941.
61. FREY-WYSSLING, A. Submicroscopic morphology of protoplasm and its derivatives. Eisevier Publ. Co. Amsterdam, New York, London. 255 pp. 1948.
62. GEE, A. H., and G. A. HUNT. Single cell technic. A presentation of the pipette method as a routine laboratory procedure. Jour. Bact. 16: 327-347. 1928.
63. GELFAN, S. The electrical conductivity of protoplasm and a new method of its determination. Univ. Calif. Publ. Zool. 29: 453-465. 1927.
64. GLASER, R. W. The germ-free culture of certain invertebrates. Chap. 7: 164-184. Reyniers, J. A. Micrurgical and Germ-Free Methods. Chas. C. Thomas. Springfield, Ill. 274 pp. 1943.
65. ———— and H. A. CORIA. The culture and reactions of purified protozoa. Amer. Jour. Hyg. 21: 111-121. 1935.
66. GORDON, H. K., and R. CHAMBERS. The particle size of acid dyes and their diffusibility into living cells. Jour. Cell. & Compar. Physiol. 17: 97-108. 1941.
67. GREEN, P. B., and G. B. CHAPMAN. On the development and structure of the cell wall in *Nitella*. Amer. Jour. Bot. 42: 685-693. 1955.
68. GUILLIERMOND, A. Observations vitales sur le chondriome des végétaux et recherches sur l'origine des chromoplastides et le mode de formation des pigments xanthophylliens and carotinians. Rev. Gen. Bot. 36: 372-416. 1919.

69. ———— Recherches ultramicroscopiques sur les cellules végétales. Rev. Gen. Bot. **42**: 129-143. 1930.
70. GUTTERBERG, H. V. 1943. The cultivation of isolated plant cells. Planta. **33**: 576-588. 1943.
71. HEILBRUNN, L. V. Studies in artificial parthenogenesis. II. Physical changes in the egg of *Arbacia*. Biol. Bull. **29**: 149-203. 1915.
72. ———— The physical effects of anesthetics upon living protoplasm. Biol. Bull. **39**: 307-315. 1920.
73. ———— Protoplasmic viscosity changes during mitosis. Jour. Exp. Zool. **34**: 417-447. 1921.
74. HEILBRONN, A. Eine neue Methode zur Bestimmung der Viskosität lebender Protoplasten. Jahr. Wiss. Bot. **61**: 284-338. 1922.
75. HEILBRUNN, L. V. The Dynamics of Living Protoplasm. Acad. Press, Inc. New York. 325 pp. 1956.
76. HILDEBRAND, E. M. Infectivity of the fire blight organism. Phytopath. **27**: 850-852. 1937.
77. ———— Techniques for the isolation of single micro-organisms. Bot. Rev. **4**: 627-664. 1938a.
78. ———— Growth rates of phytopathogenic bacteria. Jour Bact. **35**: 487-492. 1938b.
79. ———— Strains of the fire blight organism. [Abs.] Phytopath. **30**: 9. 1940a.
80. ———— Cane gall of brambles caused by *Phytomonas rubi* n. sp. Jour. Agr. Res. **61**: 688-696. 1940b.
81. ———— Micrurgical studies with special reference to the infection mechanism in tomato. [Abs.] Phytopath. **31**: 13-14. 1941a.
82. ———— Rapid transmission of yellow-red virosis in peach. Contr. Boyce-Thomp. Inst. **11**: 485-496. 1941b.
83. ———— A micrurgical study of crown gall infection in tomato. Jour. Agr. Res. **65**: 45-59. 1942a.
84. ———— A darkening technique for inducing virus symptoms in mature as well as in growing leaves. Science **95**: 390. 1942b.
85. ———— Micrurgy and botany with special reference to phytopathology. Chap. 3: 72-92. Reyniers, J. A. Micrurgical and Germ-Free Methods. Chas. C. Thomas. Springfield, Ill. 274 pp. 1943.
86. ———— Techniques for the isolation of single microorganisms. II. Bot Rev. **16**: 181-207. 1950.
87. ———— Relative stability of fire blight bacteria. Phytopath, **44**: 192-197. 1954.
88. ———— Sweetpotato internal cork virosis indexed on Scarlett O'Hara morning glory. Science **123**: 605-507. 1956a.
89. ———— Cork virus leaf spots on Triumph sweetpotato contain separated parenchyma cells. Science **123**: 1034-1035. 1956b.
90. ———— Rapid inoculation techniques for mechanical transmission of sweetpotato internal cork virus. Plant Dis. Rep. **40**: 527-530. 1956c.
91. ———— The mechanism of virus inoculation in plants. [Abs.] Phytopath **48**. 262-263. 1958.
92. ———— and L. H. MACDANIELS. Modes of entry of *Erwinia amylovora* into the principal pome fruits [Abs.] Phytopath. **25**: 20. 1935.
93. ———— C. E. Steinbauer, C. Drechsler, and E. C. Tatman. Studies on sweetpotato stem rot or wilt and its causal agent. Plant Dis. Rep. **42**: 112-121. 1958.

94. HOFMEISTER, W. Die Lehre von der Pflanzen Zelle. Leipzig. Handb. Physiol. Bot. Vol. 1: 1-404. 1867.
95. HOFMEISTER, L. Mikrurgische Studien an Diatomeen. Seits. Wiss. Mikr. 57: 259-273. 1940a.
96. ———— Studien über Mikroinjektion in Planzenzellen. Zeits. Wiss. Mikr. 57: 274-290. 1940b.
97. HOWLAND, R. B., and A. Bernstein. A method for determining the oxygen consumption of a single cell. Jour. Gen. Physiol. 14: 339-348. 1931.
98. IWANOWSKI, D. Über die Mosaikkrankheit der Tabakopflanze. Zeits. Pflanzenkr. 13: 1-41. 1903.
99. JACKSON, R. C. Low chromosome number for plants. Science 126: 1115-1116. 1957.
100. KAMIYA, N. The control of protoplasmic streaming. Science. 92: 462. 1940.
101. KASSANIS, B., and F. M. L. SHEFFIELD. Variations in the cytoplasmic inclusions induced by three strains of the tobacco mosaic virus. Ann. Appl. Biol. 28: 360-367. 1941.
102. KELLY, S. M., and L. M. BLACK. The origin, development and cell structure of a virus tumor in plants. Amer. Jour. Bot. 36: 65-73. 1949.
103. KERR, T., and I. W. BAILEY. The cambium and its derivative tissues. X. Structure, optical properties, and chemical composition of the so-called middle lamella. Jour. Arn. Arb. 15: 327-349. 1934.
104. KITE, G. L. The nature of the fertilization membrane of the egg of the sea urchin (*Arbacia punctulata*). Science (N.S.) 36: 562-564. 1912.
105. ———— The relative permeability of the surface and interior portions of the cytoplasm of animal and plant cells. Biol. Bull. 25: 1-7. 1913a.
106. ———— Studies on the physical properties of protoplasm. Amer. Jour. Physiol. 32: 146-164. 1913b.
107. ———— Studies on the permeability of the internal cytoplasm of animal and plant cells. Amer. Jour. Physiol. 37: 282-299. 1915.
108. ———— and R. CHAMBERS. Vital staining of chromosomes and the function and structure of the nucleus. Science 36: 639-641. 1912.
109. KUHNE, W. Untersuchungen über das Protoplasma und die Contractilität. Leipzig. 1864.
110. LEPESCHKIN, W. Kolloidchemie des Protoplasmas. Verlag von Julius Springer Berlin. 228 pp. 1924.
111. LISTER, A. Notes on the plasmodium of *Badhamia utricularis* and *Brefeldia maxima*. Ann Bot. 2: 1-24. 1888.
112. LIVINGSTON, L. G., and B. M. DUGGAR. Experimental procedures in a study of the location and concentration within the host cells of the virus of tobacco mosaic. Biol. Bull. 67: 504-512. 1934.
113. LURIA, S. E. General Virology. John Wiley & Sons. New York. 427 pp. 1953.
114. MCCLUNG, L. S. Recent developments in microbiological techniques. Ann. Rev. Microbiol. 1: 395-422. 1949.
115. MCWHORTER, F. P. Chlorazol Fast Pink B and Trypan Blue tests for virus and other proteinaceous inclusions. Stain Techn. 32: 135-138. 1957.
116. ———— and W. C. PRICE. Evidence that two different plant viruses can multiply simultaneously in the same cell. Science 109: 116-117. 1949.
117. MARAMOROSCH, K. Semiautomatic equipment for injecting insects with measured amounts of liquids containing viruses or toxic substances. Phytopath. 46: 188-190. 1956.

118. ————, M. K. Brakke, and L. M. Black. Mechanical transmission of a plant tumor virus to an insect vector. Science **110**: 162-163. 1949.
119. Muhlethaler, K. Electron micrographs of plant fibers. Biochim. Biophys. Acta **3**: 15-25. 1949a.
120. ———— The structure of bacterial cellulose. Biochim. Biophys. Acta **3**: 527. 1949b.
121. ———— Electron microscopy of developing plant cell walls. Biochim. Biophys. Acta **5**: 1-9. 1950.
122. ———— The structure of plant slimes. Exp. Plant Res. **1**: 341-350. 1950.
123. Nageli, O. Von. Cell nuclei, cell formation and cell growth in plants. Zeits. Wiss. Bot. **3&4**: 22-253. 1846.
124. Needham, J., and D. M. Needham. The hydrogen-ion concentration and oxidation reduction potential of the cell interior: a microinjection study. Proc. Roy. Soc. [London]. B **98**: 259. 1925.
125. Nemec, B. Über Pflanzentumoren. Arch. Zellforsch. **6**: 172-177. 1928.
126. Nichols, S. P. Methods of healing in some algal cells. Amer. Jour. Bot. **9**: 18-27. 1922.
127. ———— The effect of wounds upon the rotation of the protoplasm in the internodes of Nitella. Bull. Torrey Bot. Club. **52**: 351-363. 1925.
128. Norman, A. G. The Biochemistry of Cellulose, the Polyuronides, Lignin, etc. Oxford Clarendon Press. 237 pp. 1937.
129. Overton, E. Studien über die Aufnahme der Anilinfarben durch die lebende Zelle. Jahrb. Wiss. Bot. **34**: 669-701. 1900.
130. Peterfi, T. Mikrurgische Methodik. Handb. Biol. Arbeitsmeth. (Abderhalden). **5**: 479-516. 1923.
131. ———— Die heizbare feuchte Kammer. Leit. Wiss. Mikroskop. **44**: 296-308. 1927.
132. Pfeffer, W. Zur Kenntnis der Plasmahaut und der Vacuolen nebst Bemerkungen über den Aggregatszustand des Protoplasmas und über osmotische Untersuchungen. Abhandl. Math. Physik. Kl. K., Sachs. Ges. Wiss. **16**: 187-343. 1900.
133. Pinoy, P. E. A propos du cancer des plantes on crown gall. Compt. Rend. Acad. Sci. [Paris] **180**: 311-313. 1925.
134. Policard, A. La méthode de la microincinération, exposé practique. 50 pp. 1939.
135. Porter, K. R., A. Claude, and E. F. Fullam. A study of tissue culture cells by electron microscopy. Methods and preliminary observations. Jour. Exp. Med. **81**: 233-246. 1945.
136. Preston, R. D., E. Nicolai, R. Reed, and A. Millard. An electron microscope study of cellulose in the cell wall of *Valonia ventricosa*. Nature **162**: 665-667. 1948.
137. Pringsheim, E. G. Pure cultures of algae. Their preparation and maintenance. Cambridge Univ. Press. London, New York. 119 pp. 1946.
138. Riker, A. J., W. M. Banfield, W. H. Wright, G. W. Keitt, and H. E. Sagen. Studies on infectious hairy root of nursery apple trees. Jour. Agr. Res. **41**: 507-540. 1930.
139. Sands, H. E. The structure of the chromosomes in *Tradescantia virginica* L. Amer. Jour. Bot. **10**: 343-360. 1923.
140. ———— A microdissection of the pachytene threads of *Tradescantia virginica* L. with observations on some aspects of mitosis. Jour. Gen. Physiol. **9**: 181-190. 1925.

141. SANTIAGO, J. C. The importance of single spore techniques on rust work. Robigo **1956** (2): 11-12. 1956.
142. SCARTH, G. W. XXVI. Adhesion of protoplasm to cell walls and the agents which cause it. Trans. Roy. Soc. Canada **16**: 137-143. 1923.
143. ———— The toxic action of distilled water and its antagonism by cations. Trans. Roy. Soc. Canada **18**: 97-104. 1924a.
144. ———— Colloidal changes associated with protoplasmic contraction. Quart. Jour. Exp. Physiol. **14**: 99-113. 1924b.
145. ———— The action of cations on the contraction and viscosity of protoplasm. Quart. Jour. Exp. Physiol. **14**: 114-122. 1924c.
146. ———— The influence of external osmotic pressure and of disturbance of the cell surface on the permeability of *Spirogyra* for acid dyes. Protoplasma **1**: 204-213. 1926.
147. ———— The structural organization of plant protoplasm in the light of micrurgy. Protoplasma **2**: 189-205. 1927.
148. ———— The influence of H-ion concentration on the turgor and movement of plant cells with special reference to stomatal behavior. Proc. Int. Congr. Plant Sci. Vol. 2: 1151-1162. 1929.
149. SCHMIDT, H. D. On the minute structure of the Hepatic lobules, particularly with reference to the relationship between the capillary blood vessels, the Hepatic cells, and the canals which carry off the secretion of the latter. Amer. Jour. Med. Sci. (N.S.) **37**: 2-40. Blanchard & Lea. Philadelphia. 1859.
150. SCOTT, F. M. Internal suberization of plant tissues. Science **108**: 654-655. 1948.
151. ———— Plasmodesmata in xylem vessels. Bot. Gaz. **110**: 492-495. 1949.
152. ———— Internal suberization of tissues. Bot. Gaz. **111**: 378-394. 1950.
153. SCOTT, J. F. Electron micrograph studies on sodium desoxyribose nucleate. Biochem. et Biophys. Acta **2**: 1-6. 1948.
154. SCHLEIDEN, M. J. Beitrage zur Phytogenesis. Arch. Anat. Physiol. Wiss. Med: 137-176. 1838.
155. SCHULTZE, M. Das Protoplasma der Rhizopoden und der Pflanzenzellen; ein Beitrag zur Theorie der Zelle. Leipzig. 1863.
156. SEIFRIZ, W. Observations on the structure of protoplasm by aid of microdissection. Biol. Bull. **34**: 307-324. 1918.
157. ———— Viscosity values of protoplasm as determined by microdissection. Bot. Gaz. **70**: 360-386. 1920.
158. ———— Observations on some physical properties of protoplasm by aid of microdissection. Ann. Bot. **35**: 269-296. 1921.
159. ———— New material for microdissection. Protoplasma. **3**: 191-196. 1928.
160. ———— The viscosity of protoplasm. Bull. Nat. Res. Council. No. 69: 229-261. Washington, D. C. 1929.
161. ———— Alveolar structure of protoplasm. Protoplasma **9**: 177-208. 1930.
162. SHEFFIELD, F. M. L. The formation of intracellular inclusions in Solanaceous hosts infected with aucuba mosaic on tomato. Ann. Appl. Biol. **18**: 471-493. 1931.
163. ———— The development of assimilatory tissue in Solanaceous hosts infected with aucuba mosaic of tomato. Ann. Appl. Biol. **20**: 57-69. 1933.
164. ———— Experiments bearing on the nature of intracellular inclusions in plant virus diseases. Ann. Appl. Biol. **21**: 430-453. 1934.

165. ———— The susceptibility of the plant cell to virus disease. Ann. Appl. Biol. **23**: 498-508. 1936.
166. ———— Micrurgical studies on virus infected plants. Proc. Roy. Soc. B. **126** 528-538. 1939a.
167. ———— X. Some effects of plant virus diseases on the cells of their host. Jour. Roy. Micr. Soc. III. **59**: 149-161. 1939b.
168. SINKE, N. Experimental studies on cell nuclei. Mem. Coll. Sci. Kyoto Imper. Univ. B. **15**: 1-126. 1939.
169. SMITH, E. F., N. A. BROWN, and L. MCCULLOCH. The structure and development of crown gall, a plant cancer. U. S. Dept. Agr., Bur. Plant Ind., Bul. 255. 1912.
170. STANLEY, W. M. Chemical studies on the virus of tobacco mosaic. VI. The isolation from diseased Turkish tobacco plants of a crystalline protein possessing the properties of tobacco mosaic virus. Phytopath. **26**: 305-320. 1936.
171. STEERE, R. L., and R. C. WILLIAMS. Identification of crystalline inclusion bodies extracted intact from plant cells infected with tobacco mosaic virus. Amer. Jour. Bot. **40**: 81-84. 1953.
172. STOREY, H. H. Investigations of the mechanism of the transmission of plant viruses by insect vectors. I. Proc. Roy. Soc. [London] B. **113**: 463-485. 1933.
173. ———— Investigations of the mechanism of the transmission of plant viruses by insect vectors. II. The part played by puncture in transmission. Proc. Roy. Soc. B. **125**: 455-477. 1938.
174. ———— Investigations of the mechanism of the transmission of plant viruses by insect vectors. III. The insect's saliva. Proc. Roy. Soc. B. **127**: 526-543. 1939.
175. STRUGGER, S. Untersuchungen an isolierten Kernen der Internodial-Zellen von *Chara fragilis* Desv. Planta **8**: 717-741. 1928.
176. TAYLOR, C. V. An accurately controllable micropipette. Science **51**: 617-618. 1920.
177. ————, and D. M. WHITAKER. Potentiometric determinations in the protoplasm and cell sap of *Nitella*. Protoplasma **3**:: 1-6. 1928.
178. TILFORD, P. E. Fasciation of sweet peas caused by *Phytomonas fascians*. n. sp. Jour. Agr. Res. **53**: 383-394. 1936.
179. THOMAS, R. C. Composition of fungus hyphae. I. The Fusaria. Amer. Jour. Bot. **15**: 537-547. 1928.
180. ———— Composition of fungus hyphae. II. Sclerotinia. Amer. Jour. Bot. **17**: 779-787. 1930.
181. TOPLER, F. Über Vernarbung und Wundreis in Algenzellen. Ber. Deut. Bot. Ges. **21**: 291-300. 1903.
182. TRAGER, W. Cultivation of the virus of grasserie in silkworm tissue cultures. Jour. Exp. Med. **61**: 501-513. 1935.
183. VAN ITERSON, G. De Wording van den plantaardigen celwand. Chem. Weekblad. **24**: 166-187. 1927.
184. VIEGAS, A. P., and E. M. HILDEBRAND. Acetylene microburner for making micropipettes for micromanipulator. Phytopath. **46**: 698. 1956.
185. VON MOHL, H. Über die Verbindung der Zellen untereinander. Diss., Tubingen. 1835.
186. ———— Über die Saftbewegung im Innere der Zelle. Bot. Zeit. **4**: 73-78, 89-94. 1846.
187. WADA, B. Anstichversuche an der Zellen der Staubfadenhaare von *Tradescantia virginica*. Cytologia **1**: 404-416. 1930.

188. WRIGHT, W. H. A convenient case for sterilizing and storing micropipettes. Jour. Bact. **17**: 10. 1829.

189. ———— and E. F. MCCOY. An accessory to the Chambers' apparatus for the isolation of single bacterial cells. Jour. Lab. & Clin. Med. **12**: 795-800. 1927.

190. ———— and A. A. HENDRICKSON, and A. J. RIKER. Studies on the progeny of single cell isolations from the hairy root and crown gall organisms. Jour. Agr. Res. **41**: 541-547. 1930.

191. WYCKOFF, R. W. G. The electron microscopy of developing bacteriophage. I. Plaques on solid media. Biochim. et Biophys. Acta **2**: 27-37. 1948.

192. ———— Electronmicroscopy. Technique and Application. Interscience Publ., Inc. New York. 248 pp. 1949.

ABSORPTION OF WATER BY PLANTS

R. O. SLATYER

Division of Land Research and Regional Survey,
C.S.I.R.O., Canberra, A.C.T., Australia.

Introduction 332

Terminology and Units 333

Processes and Mechanisms 336

Active Absorption 337

Passive Absorption 340

Factors Affecting Water Absorption 344

Supply of Water at the Root Surface 345

Entry of Water into the Root 350

Passage of Water in Conducting Elements 357

Movement of Water Through and Out of Leaves 359

Relative Importance of Factors Affecting Absorption 362

Absorption of Water by Leaves and Other Aerial Organs 367

Probable Mechanisms Involved 367

Significance of Absorption in Nature 373

General Summary 375

Literature Cited 377

INTRODUCTION

This review has been prepared as a supplement to Kramer's (1945a) paper, published in this journal. The intervening period has seen many old controversies renewed, particularly the osmotic versus non-osmotic theories of active absorption and the significance of water absorption by plants directly through their aerial organs. At the same time some aspects have received relatively little attention, particularly those concerned with the influence of soil physical conditions on absorption. Over the period several general reviews hav been prepared. Among these may be listed the two texts by Kramer (1949) and Crafts, Currier and Stocking (1949); the monogram on soil physical conditions and plant growth, under the general editorship of Shaw (1952); and the recent Encyclopedia of Plant Physiology, under the general editorship of Ruhland, of which Volumes I-III (1955, 1956a, 1956b) are of direct relevance. In addition to these valuable sources of reference, numerous reviews on specific aspects of water absorption have been prepared and the proceedings of several symposia have been published, all of which are cited in the text.

In some respects this relative abundance of material has tended to make the reviewer's task difficult, since it necessitates a different type of presentation in order to avoid unnecessary repetition. For this reason special emphasis is placed on the relative importance of the various factors affecting absorption, and these are evaluated in terms of the van den Honert (1948) hypothesis which regards the movement of water into, through and out of the plant as a catenary process, the rate of which is effectively controlled at the source of greatest resistance along its path. The implications of this hypothesis and the significance of the factors affecting absorption are discussed in detail.

Where possible, the material cited has been from papers published since Kramer's earlier paper was prepared, although in many cases earlier material has had to be referred to with reference to specific matters. Not only has the method of treatment deviated from the earlier paper; the subject matter has also been altered to some extent. In particular, some aspects of root pressure and guttation have not been considered, and a chapter on absorption through aerial organs has been added. The literature coverage pertaining to this review was completed in December, 1959.

TERMINOLOGY AND UNITS

At the present time, a variety of terms and units are utilised for discussions of absorption of water by plants. All of these are derived, in one form or another, from the fundamental thermodynamic principle concerning the movement of materials, that each molecule possesses a total internal energy equal to the sum of its internal kinetic and potential energies, and that the system is subject to spontaneous change if there is any process whereby the internal energy of the constituent molecules can be effectively reduced.

The terminology in most common use is that of Meyer (1938, 1945, 1956) which regards water movement as tending to occur along gradients of decreasing diffusion pressure. The basic unit involved is diffusion pressure deficit (DPD) which is defined by Meyer as the amount by which the diffusion pressure of water in a given state is less than that of pure water at the same temperature and under atmospheric pressure. The DPD difference (DPDD) between any two points is a measure of the driving force, so that the tendency of water to move, for example, inward into a plant or plant cell, can be expressed as the difference between the algebraic sum of the DPDs associated with internal factors and the algebraic sum of the DPDs associated with external factors, thus :–

$$DPDD = (\Sigma DPD)\,i \quad -(\Sigma DPD)e$$

Meyer considers DPD to be an equivalent pressure directly related to the direction of transfer of water through a plant system, and the concept can be extended to include soil-plant water movement, since the commonly used soil water units, soil moisture tension and total soil moisture stress (TSMS), are identical in concept and dimensions with the DPD of soil water. In this case, tendency for water movement into a plant can be expressed by changing the last term of the above equation to TSMS.

Although this terminology has had general acceptance among plant workers, there has been a tendency in recent years to utilise alternative terms and units. This has been motivated, in part, by the fact that the use of pressure terms and units can be misleading. For instance, high equivalent pressures or suctions can frequently be measured in soil or plant water systems, but these values represent potential energies and do not necessarily imply the existence of actual pressure differences. Furthermore, the simple and straightforward equation re-

lating DPD to osmotic pressure and turgor pressure (DPD = OP–TP) refers ideally to the completely vacuolated plant cell. In tissues in which the non-vacuolar volume reaches significant proportions, additional factors may contribute to the free energy status of the water in the system.

Among the studies which have considered alternative treatments may be cited those of Edlefsen (1941) who provided a generalized analysis of water absorption; of Edlefsen and Anderson (1943) who extended this analysis with more emphasis on the movement of soil water; and of Broyer (1947a, 1947b, 1950, 1951a, 1951b) who presented a series of valuable and lucid interpretations of the free energy function with special reference to the movement of water into, and within, the plant.

Edlefsen (1941) suggested the function "specific free energy" to describe the thermodynamic state of water in plant or soil. He proposed that the total specific free energy at any point in the soil-plant-atmosphere system is composed of several appropriately defined component free energies. These arise from the free energy possessed by the water because of the hydrostatic pressure it is under, because of the presence of dissolved material, because of its presence in a force field such as a gravitational or adsorptive field, or due to interfacial surface tension.

Broyer (1947a) defined "net influx specific free energy" (NIF) as the difference in action capacity between the algebraic sum of the specific free energies tending to cause water to move into the system and those tending to cause water to move out of the system. Thus the net influx specific free energy is equal to the sum of the influx specific free energies (ΣIF) diminished by the sum of the efflux specific free energies (ΣEF) as under :–

$$NIF = \Sigma IF - \Sigma EF$$

The principal partial specific free energies concerned in the movement of water through the plant are identified by Broyer as hydrostatic specific free energy, osmotic solute specific free energy, non-metabolic specific free energy and metabolic specific free energy. It is apparent that, while Broyer's NIF is analagous in form and application to Meyer's DPDD, not all the partial specific free energies contributing to NIF can be interpreted through diffusion pressure concepts. As a result, DPD and DPDD are only partially represented in the concept of NIF.

An alternative method of expression is in terms of the chemical potential of water, which is identical with the partial molal free energy. This procedure was adopted by Day (1942) who referred to it as "moisture potential." Schofield, cited by Owen (1952), suggested the term "water potential" for the same function, and more recently the 1959 UNESCO conference on plant water relations in arid regions favourably considered a terminology based on an evaluation of this function.

These considerations have been outlined by Slatyer and Taylor (in press). Briefly, the aim was to develop a terminology in which the primary components of the existing "hydraulic" terminologies can be identified but in which a more comprehensive description can be made of the state of water in the plant and soil system. Thus, the "water potential" is considered to comprise an osmotic potential, due to the concentration of solutes, analogous to the osmotic pressure term in common use; a pressure potential which can be identified with turgor pressure (actually, the pressure potential equals turgor pressure multiplied by the specific volume of water, which is unity) or hydraulic pressure in saturated soils; and a matric potential, which evaluates the water release relationship. In soils this is associated with soil suction or soil moisture tension; in plants it describes the condition of water within the cell walls or the colloidal system of the protoplasm, a factor which is not accounted for by turgor pressure or osmotic potential. As systems become more complex, and more independent variables are needed to describe the status of water, additional terms may be introduced so that they can be adequately described and interpreted. Likewise, external force fields, such as those of gravity, can be accounted for.

The question of units of expression is one of importance and interest. DPD is almost universally expressed as atmospheres; specific free energy is generally expressed in ergs.gm^{-1}, although Broyer uses volumed specific free energies expressed as litre atmospheres.litre^{-1} or atmospheres. The atmosphere is traditionally a unit of pressure and is used in this context by Meyer, since he regards DPD as a pressure unit. Since pressure is equal to energy per unit volume, it can be argued that to use atmospheres as energy units is valid but at the same time it must be recognized that to use an established pressure unit to express energy data can easily lead to confusion. After the procedure of Buckingham (1907), Owen (1952) suggested that water potential might be expressed in units of gravitational potential, as centimetres or metres

of water. Such procedure provides a unit which is simple and can be made quantitatively and numerically comparable to the common pressure unit of atmospheres.

The terms and units which are adopted by individual researchers for the study of water absorption largely reflect their taste and training, but there is much to be said for the adoption by physiologists of more basic thermodynamic terminology. Several factors have contributed to the slow transition of thought to this more basic system. There has been a lack of direct methods for measuring free energy with convenience and precision, which has resulted in the expression of results in terms of equivalent pressures (pressure frequently being the component free energy applied to result in an equilibrium condition of zero movement). However, this has now been largely overcome by the application of hygrometric techniques to measurements of water potential (Spanner, 1951; Monteith and Owen, 1958; Richards and Ogata, 1958; Korven and Taylor, 1959). There is also the fact that the thermodynamic terminology is more complicated, so that scientists without training in physical chemistry are reluctant to use it. Moreover, the concept of DPD and of DPD gradients is widely accepted at the present time, and, since it is satisfactory for most purposes, there is a widespread tendency to retain it and to extend it, where necessary, to cover water movement beyond the plant (Meyer, 1956). In the present paper thermodynamic terms and units based on the chemical potential of water have been adopted. The term "water potential" is therefore used in place of DPD with reference to the free energy of plant water and in place of TSMS with reference to the free energy of soil water. "Osmotic potential" is used instead of osmotic pressure. Turgor pressure is retained when it is used as a pressure, but is referred to as a pressure potential where appropiate. The units of expression are $ergs.gm^{-1}$.

PROCESSES AND MECHANISMS

Renner (1912, 1915) distinguished between the two main groups of processes involved in the absorption of water by plants, on the basis that "active" absorption is associated with conditions maintained by actively metabolizing root cells, and "passive" absorption results from transpirational activity within the shoot. This division appears logical and satisfactory, since not only are the energy sources different in nature and origin, but under most conditions the two processes appear to be independent and do not operate at the same

time. Thus, when atmospheric conditions favour very slow or negligible transpiration and little soil water stress exists, there is little tension in the plant system and active absorption proceeds. On the other hand, when the general stress level in the plant is high, as a result of either rapid transpiration or dry soil, or both, active absorption does not operate and passive absorption is the primary sorption mechanism.

Quantitatively passive absorption is of far greater significance than active absorption, and most water is absorbed by the plant in this manner. Active absorption finds expression mainly during nocturnal hours in the form of root pressure and guttation.

ACTIVE ABSORPTION

The recent controversy as to whether or not active absorption involves non-osmotic mechanisms has been due, in the main, to the fact that, although most absorption can be explained on the basis of simple osmotic theory, some aspects cannot adequately be explained in this manner.

In support of the osmotic theory, Eaton (1943) produced data which show that the amount of exudation from detopped cotton plants is proportional to the difference between the osmotic potential of the xylem sap and that of the solution surrounding the roots. This evidence confirmed and extended the results of Kramer (1941) who had found that rapid reversal from exudation to absorption through the stump could be demonstrated by transferring the roots to and from water and sucrose solutions. This behaviour could be produced in less than a minute and appeared to be similar to the responses of a simple osmometer.

Arisz, Helder and van Nie (1951) also concluded that active absorption is controlled by a diffusion or free energy gradient. They considered that absorption follows the active transport or secretion of salts into the xylem, and that, although development of the gradient involves energy expenditure through metabolism, the movement of water is primarily influenced by the slope of the gradient developed and the permeability of the tissue. These workers confirmed the results of Eaton (1943) that the concentration of the external solution is inversely proportional to the rate of exudation and directly proportional to the concentration of the exudate. Similar results were obtained by van Andel (1952). In general, workers supporting the simple osmotic explanation of active absorption agree with Levitt's (1954a)

conclusions that negative gradients do not exist between the water-absorbing roots of plants and their environment and that experimental evidence claiming to prove that active water absorption is a non-osmotic phenomenon is due to error in determination or in interpretation of results.

Opposing this viewpoint, van Overbeek (1942) found that the osmotic concentration of the exudate from detopped tomato plants is significantly lower than the concentration of the external medium at which exudation ceases. Evidence for a non-osmotic factor in water absorption has also been obtained from other experiments. Bennett-Clark, Greenwood and Barker (1936) found that the osmotic potential of certain tissues is higher when measured plasmolytically than when measured cryoscopically on the expressed sap. This was regarded as evidence that the protoplasm secretes water into the vacuole instead of acting as a passive, differentially permeable, membrane. Similar discrepancies were noted by other workers (Mason and Phillis, 1939; Roberts and Styles, 1939; Bennett-Clark and Bexon, 1940; Currier, 1944), but, because of the possible inherent errors in the osmotic potential measurements, Levitt (1947) considered this evidence to be inconclusive.

Levitt (1947) also calculated the energy needed to maintain absorption gradients through respiration and concluded that the maintenance of a gradient of more than 2.10^6 ergs.gm^{-1} would rapidly deplete the carbohydrate reserves of a plant. Levitt's assumption of the need for respiration to provide the energy for non-osmotic absorption appears valid, as there is considerable evidence to link the processes, and water intake is reduced by factors which tend to inhibit respiration (Steward, Stout and Preston, 1940; van Overbeek, 1942; Rosene, 1944, 1947, 1950; Kelly, 1947; Hackett and Thimann, 1952; Bonner, Bandurski and Millerd, 1953). However, his conclusions were questioned on the basis that the permeability values used were incorrect and the thermodynamic calculation wrongly based (Bennett-Clark, 1948; Meyers, 1951; Spanner, 1952). A lively controversy commenced on these issues (Levitt, 1953, 1954b; Spanner, 1954), and Stiles (1956) considers this aspect of the question to be still open.

Active absorption of water is closely related to respiration, and this relationship, in turn, appears to be closely linked with auxin-induced water uptake (Reinders, 1938, 1942; Hackett and Thimann, 1952). In general, it was noted that auxin significantly promotes water absorption and that this absorption is inhibited by respiratory inhibi-

tors. The latter authors, in common with Ketellapper (1953), also found that the increased water uptake is accompanied by a decrease in the osmotic potential of the cell sap and concluded that the effect of auxin on water uptake is not due to the formation of osmotically active substances in the cell. Hackett and Thimann (1952, 1953) subsequently concluded that the effect of auxin is on the cell wall, since respiratory inhibitors have a parallel effect on water uptake and on cell enlargement. They also showed that the effect of auxin on respiration parallels its effect on water uptake, which provides further evidence of the role of metabolic processes in active absorption.

Bonner, Bandurski and Millerd (1953) demonstrated that in the presence of auxin water absorption could take place, although at reduced rate, from mannitol solutions hypertonic to the cell sap. Again a close relationship between uptake and respiration was demonstrated throughout the range of solutions tested. However, this strong evidence of non-osmotic uptake, with respiration as a suggested energy source, was later found to be in error (Burström, 1953; Ordin, Applewhite and Bonner, 1956). These workers showed that in the presence of mannitol the osmotic potential of the cell sap increases rapidly and in approximate proportion to the concentration of added mannitol. They therefore concluded that a negative osmotic gradient does not exist and that it is unnecessary to invoke a non-osmotic mechanism to explain water uptake in these experiments. Although Mercer (1955) questioned Burström's (1953) interpretations on the basis that the increase in osmotic potential of the cell sap probably occurs as a result of decrease in hydration instead of permeation of mannitol, the conclusion remains valid that water uptake is osmotic. Mercer (1955) also pointed out that many of the experiments measuring water uptake by changes in tissue weight are subject to error because once the cells become plasmolysed the tissue weight fails to respond to changes in the volume of the protoplasts, i.e., to changes in apparent osmotic volume. For this reason the data of van Overbeek (1942) also lose their significance as evidence of non-osmotic absorption.

The mechanism by which auxin increases water uptake has been studied by numerous workers. Some of the earlier investigators attributed the effect to increased accumulation of osmotically active substances in the cell through starch hydrolysis or salt accumulation (Reinders, 1938, 1942; Commoner and Mazia, 1942; Commoner, Fogel and Muller, 1943), but later studies which found no increase in osmotic potential of the cell sap, in fact a decrease due to cell

enlargement, showed this hypothesis to be in error (van Overbeek, 1944; Levitt, 1947; Hackett, 1952; Brauner and Hasman, 1952).

Three main effects of auxin have been suggested — increase in permeability of cell membranes, increased extensibility and plasticity of the cell wall, and direct stimulation of non-osmotic absorption. Brauner and Hasman (1949, 1952) concluded that the primary effect is to cause increased permeability and extensibility of the cell wall. A similar conclusion was reached by Levitt (1953) and Ordin, Applewhite and Bonner (1956), and this has the important corollary that auxin-induced water uptake follows osmotic principles.

Autonomic rhythms, which are frequently noticed in exudation of detopped root systems (Grossenbacher, 1939; Hagan, 1949), are sometimes cited as evidence of a non-osmotic control of active absorption. It appears probable that in these cases the endogenous rhythms are related to changes in respiration and other metabolic activity (Hagan, 1949). Van Andel (1953) found that water permeability and salt transport to the xylem varied with rate of exudation, and it is possible that these effects could also be interpreted through osmotic theory.

In conclusion, it appears that with present knowledge it is difficult to identify a non-osmotic factor in active water absorption, even though it is apparent that active absorption is closely linked to, and immediately affected by, changes in rate of respiration. As Kramer (1956b) pointed out, the fact that active absorption is linked with respiration does not prove that the process is non-osmotic. Even if it is an osmotic process, it will be affected by the permeability of the protoplasmic membranes and the concentration of solutes in the xylem—both of which are dependent on release of energy through respiration.

PASSIVE ABSORPTION

Of the processes involved in the absorption of water by plants, the most important is that which is initiated and controlled by transpiration from the shoot. It is generally attributed to evaporation of water from the mesophyll cells of the leaves, causing reduction of cell volume, which results in a decrease of water potential. This establishes a water potential gradient into these cells from the xylem of the leaf veins. Removal of water from the xylem reduces pressure on the water in the conducting system, and this reduction of pressure is transmitted through the xylem elements to the roots. In turn, a

gradient develops across the root cortex and into the soil. Thus a water potential gradient is established extending from the soil water surrounding the roots, through the plant and into the atmosphere, and water tends to move along this gradient in the transpiration stream. While this explanation oversimplifies some of the processes involved, it serves as a model for more detailed discussion.

The operation of this process involves the cohesion theory of sap ascent. The literature on this subject has recently been reviewed by Greenidge (1957), his primary conclusion being that, although weaknesses in the theory exist and many aspects of the original theory are invalid, no satisfactory alternative mechanism has yet been proposed. Several recent studies, in particular, have demonstrated that the classical cohesion theory is inadequate. For instance, it has been shown that sap ascent has continued, virtually unimpeded, despite deep and overlapping incisions into the stem made from opposite sides of a bole (Elazari-Volcani, 1936; Preston, 1952; Greenidge, 1955a, 1955b), and Greenidge (1958) found that rate of dye movement up the stem of a number of tree species was virtually unaffected even when the bole had been severed. In addition, Scholander, Love and Kanwisher (1955) and Scholander, Ruud and Leivestad (1957) found that when vine or liana stems are severed and the plants placed in air, absorption continues when the stem is again placed in water, though at a reduced rate, and leaf turgor is regained. Introduction of copper sulphate to the absorbed liquid, in toxic concentrations, did not prevent water from being absorbed and transferred above atmospheric height. It can be appreciated that, while these experiments throw doubt on the cohesion theory as originally proposed, they also demonstrate that active processes are of little importance in sap ascent so that a predominantly passive process is involved. Certainly the evidence of continuous freely mobile sap columns, of tension in the stem during transpiration, and the transport of water through metabolically inert plants, are in accord with the broader aspects of the cohesion theory. In any case, from the viewpoint of the current discussion, perhaps the best evidence that absorption is controlled by transpiration is the close relationship which exists between these two processes (Lachenmeier, 1932; Kramer, 1937, 1938; Weatherley, 1951).

The whole pathway of water movement from soil to atmosphere is lucidly interpreted by considering the soil-plant-atmosphere system as a thermodynamic continuum. This procedure has been adopted, using different viewpoints, by Gradmann (1928) and van den Honert

(1948), and by Edlefsen (1941). The former authors applied an analog of Ohm's law to water transport, which implies that water transport across any part of the system is governed by the potential difference across the segment concerned and the resistance in between. On this basis van den Honert (1948) demonstrated that resistance to water movement across the gaseous phase from leaf cells to the free atmosphere, even with open stomates, is by far the greatest resistance encountered in the movement of water through a plant freely supplied with soil water. Edlefsen (1941) drew the similar conclusion that in transpiring plants the greatest free energy drop occurs in the region between the leaf cells and the outside air, even when the soil is as dry as the permanent wilting percentage.

These studies emphasise the importance of the factors controlling transpiration in the control of absorption. Notwithstanding this, however, the lag of absorption behind transpiration will be determined by the sum of resistances in the path of water movement. In dry soils this lag is caused primarily by lack of availability of water at the root surfaces, occasioned by inadequate water supply or the reduced potential of the soil water (Kramer, 1949; Richards and Wadleigh, 1952; Philip, 1957a), but in culture solutions or relatively wet soils the primary resistances are probably in the root (Kramer, 1956d). This is confirmed by studies in which it has been demonstrated that the absorption lag is reduced if the roots are detached (Kramer, 1938), and the association of the resistances with the living cells of the root is indicated by the inhibition of absorption which follows application of metabolic inhibitors (Crafts, Currier and Stocking, 1949; Kramer, 1955b, 1956a, 1956c) and by the increased absorption which follows death of the roots (Renner, 1929; Kramer, 1932; Brouwer, 1954; Ordin and Kramer, 1956; Mees and Weatherley, 1957b).

Although the factors affecting absorption will be discussed in detail in later chapters, it is pertinent at this stage to consider the pathway and mode of movement of water across the root. Several interesting studies have been conducted on this subject in recent years, using isotopically labelled water. With oat coleoptiles, Ordin and Bonner (1956) found that the cell walls presented a greater barrier to water movement than the cytoplasm, although the reverse situation was noted for bean roots (Ordin and Kramer, 1956), a tissue which was, in any case, much more permeable to water. Subsequently, Philip (1958b,c) and Bonner (1959) showed that in both previous experiments the time course of water diffusion through the tissue has fol-

lowed closely the expectation based on resistance being uniform throughout. The only major barrier to diffusion appeared to be the cuticle, and in the tissue under study most of the exchange was presumably through the cut surfaces of the tissue cylinders.

The recent studies of Mees and Weatherley (1957a, 1957b) are of wider significance. These authors proposed that two permeability coefficients be considered in discussing the mechanism of water movement across the cortex, one being an index of permeability in relation to gradients of osmotic potential and the other to gradients of hydrostatic pressure. By establishing known osmotic and hydrostatic gradients across the root they were able to measure the change in flux per unit change in gradient and so evaluate the different coefficients. If the structure of the root had permitted diffusion only, the two coefficients should have been equal and a certain rate of water movement should have been caused by a given gradient of water potential, regardless of whether it was of osmotic or hydrostatic origin. The results obtained, however, showed that the rate of movement caused by a hydrostatic gradient is much greater than that caused by an equivalent osmotic gradient. This was attributed to two phenomena—firstly, that the osmotic permeability coefficient is increased considerably by the application of a hydrostatic gradient; secondly, that a mass flow component of water movement occurs in addition to diffusional movement.

Kramer (1932, 1940b) also found marked increases in water movement across roots when pressure gradients were applied, but, because of the absence of osmotic data, the comparative effects of osmotic and hydrostatic gradients are not known. Other workers have demonstrated that transpirational tensions result in increased root permeability (Brewig, 1936a, 1936b, 1939; Brouwer, 1953, 1954). Brouwer concluded that this reduction in resistance is due to decreased turgor pressure in the cells, which reduces the compression of the cytoplasm. Myers' (1951) conclusion that plasmolysed cells are more permeable than unplasmolysed cells supports this contention, although his data are in opposition to those of Levitt, Scarth and Gibbs (1936) and Aykin (1946). Mees and Weatherley (1957b), whose hydrostatic gradients were induced by externally applied pressure, rather than by tension, considered that the explanation may lie in the fact that reduced cell volume alters the permeability through differential extension of cell wall and cytoplasm. Dehydration alone normally reduces permeability (Kramer, 1955a), and the results of those workers who have noted reduced permeability when the osmotic potential of the external

medium increases (Aykin, 1946; Arisz, Helder and van Nie, 1951; Mees and Weatherley, 1957b) support this point.

Apart from the effect of tension on the osmotic permeability coefficient, Mees and Weatherley's (1957b) evidence for a mass flow component is of special interest. They concluded that the mass flow pathway is probably located in the cell walls and most of the cytoplasm, thus comprising most of the free space (Briggs, 1957). The fact that they observed a 90 per cent reduction in the flux through the root due to metabolic inhibition is not inconsistent with this hypothesis, nor is the fact that the flux increased if the roots were killed. The cell walls are intimately connected with the underlying cytoplasm, and active aerobic metabolism appears essential for the maintenance of low resistance in this pathway. Although no studies of this type are available for movement of water across the leaf, it is possible that there is also a mass flow component to water movement across the leaf mesophyll. Water movement through the walls certainly occurs in this tissue (Strugger, 1943, 1949; Steubing, 1949).

FACTORS AFFECTING WATER ABSORPTION

In the following paragraphs, primary attention is paid to those factors affecting passive absorption, and in this regard van den Honert's (1948) treatment of water transport in plants has particular relevance.

As mentioned previously, van den Honert, following Gradmann's (1928) earlier analysis, applied an analog of Ohm's law to water transport considering the flux of water across any part of the soil-plant-atmosphere system to vary proportionately with the potential difference and inversely with the resistance to flow.

Van den Honert regarded water transport through the plant as a catenary process, in which the rate of the slowest partial process governs the velocity of the whole. This implies that the source of the greatest resistance to movement is the overall source of control, and that resistance elsewhere is of secondary importance.

Although van den Honert did not examine water movement through the soil to the root surface, or consider soils significantly drier than field capacity, these features can readily be included in the situation, as the whole path from soil to atmosphere forms a thermodynamic continuum, to points along which water potential or specific free energy values may be assigned, and for each segment of which the loss of free energy may be related to the flux of water and the resistance

to the motion. This analysis can be applied strictly only to isothermal situations. Where temperature gradients exist, water movement can sometimes be observed in direction opposite to the gradient of water potential (Taylor and Cavazza, 1954). This phenomenon is not usually a factor of importance in the normal pattern of water absorption by transpiring plants but must always be borne in mind in non-isothermal situations.

From these considerations it can be seen that the primary factors affecting absorption are those which are responsible for the resistances developing along the transpiration path. The following discussion is directed towards an understanding of these factors and the extent to which they are significant.

SUPPLY OF WATER AT THE ROOT SURFACE

Two main phenomena are concerned with the supply of soil water to the surface of a root, namely, movement of the water to the root surface, and growth of the root into the soil mass.

RATE OF WATER MOVEMENT IN UNSATURATED SOILS. As water content decreases from saturation, rate of water movement decreases rapidly. Numerous studies have been made on this subject by soil physicists, and in recent years most aspects have been elucidated (Childs and Collis-George, 1948, 1950; Richards and Moore, 1952; Richards and Wadleigh, 1952; Klute, 1952; Staple and Lehane, 1954; Philip, 1954, 1955, 1957a, 1957b, 1958a). Philip (1957a) stated that, as water content decreases, unsaturated permeability decreases for the following reasons: (i) the total cross sectional area available for flow decreases with decreasing water content; (ii) as water content decreases, the largest pores are emptied first; since the contribution to permeability per unit area varies as the square of the pore radius, permeability may be expected to decrease much more rapidly than water content; (iii) as water content decreases, the chances of water occurring in pores or wedges, isolated from the general system of water films and channels, increases; once continuity breaks down there can be no flow in the liquid phase. In earlier years it was thought that unsaturated permeability would become zero as soil moisture tension approached a value equivalent to $1.0.10^6$ dynes.cm^{-2} (Richards, 1936; Richards and Wilson, 1936), but the investigations reported above indicate that some movement still occurs at much higher tensions. Experimental evidence supports this contention (Wilson and Richards,

1938; Richards and Weaver, 1944), the latter authors noting that, even at tensions equivalent to $1.0.10^8$ dynes.cm^{-2}, water movement could be achieved by application of pressure differences.

The proportion of vapour movement in the total movement of water increases as water content falls and probably constitutes the major proportion of water transfer in dry soils. Philip (1955, 1957b) has extended his work to include isothermal movement in the vapour and adsorbed phases, and Philip and deVries (1957) have examined the influence of temperature on water movement. This latter study included the interaction of vapour and liquid phases in total transfer of water and the differences between average temperature gradients in the air-filled pores and in the soil as a whole. Because of these considerations, neglected by some previous authors, good agreement has been obtained between experimental and theoretical data for water movement in response to temperature gradients. The extent of this movement can be quite appreciable (Edlefsen and Bodman, 1941; Hilgeman, 1948; Gurr, Marshall and Hutton, 1952; Taylor and Cavazza, 1954; Rollins, Spangler and Kirkham, 1954). As an example, the results of Hilgeman (1948) showed that, in a field study on bare soil in Arizona, a total movement of 9.8 inches of water in the top 8 feet of soil occurred over a period of 22 months.

An important aspect of vapour movement may arise if a vapour gap develops between root and soil. Philip (1955, 1957a) has shown that steep water gradients may develop near the root surfaces during transpiration and that, as a result, it is possible, even at fairly high mean soil water levels, for the soil immediately adjoining the absorbing surface to become so dry that the final transfer of water to the root could take place in the vapour phase. The discontinuity could be accentuated by soil and, more particularly, by root shrinkage during dehydration. Under these conditions absorption of water by the plant could be limited by the rate and extent of vapour transfer across the root-soil gap. A further effect of this discontinuity would be the accumulation of solutes at the evaporating soil surface. This would have two undesirable effects through a reduction in the free energy of the soil water and a cessation of nutrient absorption. The vapour gap hypothesis has been supported by Bonner (1959) but disputed by Bernstein, Gardner and Richards (1959) on the grounds that it is not necessary to invoke a vapour gap to explain observed phenomena and that, in fact, rates of vapour transport are inadequate to supply the amounts of water required.

RATE OF ROOT GROWTH INTO THE SOIL. Rates of water movement in unsaturated soils are slow, hence rate of root growth into the moist soil mass is an important agency in enabling the plant to obtain an adequate supply of soil water. This is particularly true of newly established plants for which rapid root growth is essential for survival. In such cases expansion of the absorption zone is observed to increase with increasing age and root development of the plant (Davis, 1940; Russell, Davis and Blair, 1940; Russell and Danielson, 1956). With established plants, such as orchard crops, a permanent and extensive root system exists and water extraction throughout the soil is more even (Hendrickson and Veihmeyer, 1929, 1934, 1942). In such cases maintenance of an adequate area of young and active roots and root hairs is more important than expansion of the whole root zone.

The factors which affect root growth are similar to those affecting plant growth generally—supply of growth materials and maintenance of a high degree of hydration—but in addition, temperature conditions, aeration and mechanical impedance are important factors. A discussion of the factors affecting growth materials in the form of mineral nutrients and photosynthetic products is beyond the scope of this paper. Only the effect of soil physical conditions will be mentioned here.

In general, it can be anticipated that root elongation decreases as water potential decreases and will cease when the water potential of the root tissue is reduced to the stage at which the turgor pressure in the enlarging cells reaches zero, just as stem elongation and normal cell enlargement usually cease at this point (Furr and Reeve, 1945; Blair, Richards and Campbell, 1950; Clements, Shigeura and Akamine, 1952; Ordin, Applewhite and Bonner, 1956; Slatyer, 1957a, 1957b). This implies that root extension will cease at approximately the same tissue water potential value as that at which permanent wilting occurs, as Slatyer (1957b) has shown that permanent wilting can be expected when the tissue water potential equals the osmotic potential of the cell sap. However, the fact that there is usually a gradient of osmotic potential through the plant (Stocking, 1956) may mean that root extension can be expected to cease at a water potential value slightly higher than that necessary to cause permanent wilting.

This general association of cessation of root extension with zero turgor pressure has been supported experimentally, several authors having shown that root growth is progressively inhibited with decreasing tissue water potential and ceases at approximately the permanent wilting percentage (Reed, 1939; Kaufman, 1945; Gingrich and

Russell, 1956, 1957). On the other hand, since the elongating zone of the root consists of incompletely vacuolated tissue, it cannot be expected that root extension will always follow these osmotic principles. In fact, some observations have shown that root growth may continue at much lower water potentials than are required to inhibit hypocotyl elongation (Ronnike, 1957). Also, if some roots are in moist soil, it is possible that the water potential through the root system will be reduced less than if all the roots are in dry soil, and extension may proceed even in those roots which are situated in the drier parts of the soil profile. This, too, is generally supported by experimental evidence (Breazeale and Crider, 1934; Hunter and Kelley, 1946; Volk, 1947; Kmock, Ramig, Fox and Koehler, 1957), although some investigators have not been able to demonstrate it (Hendrickson and Veihmeyer, 1931).

The association of cessation of root extension with permanent wilting is to be expected from the indirect effects of wilting on the supply of growth materials through photosynthesis and mineral absorption. It helps to explain why some investigators have observed that absorption of soil water appears to cease at the permanent wilting percentage (Veihmeyer and Hendrickson, 1927, 1928, 1934, 1949; Hendrickson and Veihmeyer, 1929, 1945). As Slatyer (1957b) has pointed out, there is no essential physical reason why absorption should stop at the permanent wilting percentage, but if root extension ceases as a result of decreasing water potential and water movement through the soil to the root is very slow, it is clear that absorption will be severely inhibited and for practical purposes could be considered as having stopped.

The effect of soil temperature on root growth is similar in most respects to the influence of temperature on growth generally (Hagan, 1952a). Within limits, increasing temperature results in increased cell division and cell elongation (Burström, 1941; Stuckey, 1941; Brown and Rickless, 1949); temperature is also of influence in the supply of other essential growth materials such as carbohydrates, mineral nutrients and water (Kramer, 1949; Hagan, 1952a). Optimal soil temperatures for root growth vary with species and the pre-conditioning of the plant, but for most species little root growth takes place below 5°C or above 40°C (Hagan, 1952b).

The effect of aeration on root growth is closely linked with the effect of temperature. With increasing temperature, the oxygen requirements for normal root growth increase rapidly. Cannon (1925) attributed

this feature to the decreasing solubility of oxygen in the soil solution with increasing temperature, but increase in rate of respiration is probably more important in necessitating increased oxygen supply (Russell, 1952). Increased concentration of CO_2 likewise suppresses root growth (Whitney, 1942; Erickson, 1946; Leonard and Pinckard, 1946), and this effect appears closely related to the direct effect of CO_2 on aerobic respiration. In general, it appears that in most soils CO_2 concentration is seldom high enough to damage the roots of the majority of plants, but that the concentration of oxygen may frequently be too low for optimum growth (Kramer, 1949; Harris and van Bavel, 1957). Structural characteristics affecting oxygen diffusion are therefore of primary importance (Monselise and Hagin, 1955; Bertrand and Kohnke, 1957).

The effects of mechanical impedance on root growth are, in turn, closely related to those of aeration, both factors usually being operative at the same time if impedance is due to soil density. If, on the other hand, impedance is due to low soil moisture, aeration is usually optimal when soil water content is lowest. The apparent density of the soils is an important factor in preventing root elongation; and in most soils with an apparent density of greater than 1.9, no roots of any description are found (Veihmeyer and Hendrickson, 1946, 1948). These authors also noted, as would be expected, that a lower apparent density in fine textured soils had the same inhibiting effect as a high apparent density in coarse textured soils. As they found this effect to be independent of aeration, the size of the voids in the soil appears to be a more critical factor than density alone, except where density values are extreme. This hypothesis is confirmed by the work of Gill and Miller (1956) and Wiersum (1957).

The relative importance of water movement through the soil mass to the root and of root growth into untapped soil reserves, in determining the rate of supply of water at the root surface, depends on several factors, the primary ones being soil water content, soil water potential, root activity and root density. In soils drier than field capacity, it has been demonstrated (Hendrickson and Veihmeyer, 1931, 1941; Aldrich, Work and Lewis, 1935; Veihmeyer and Hendrickson, 1938; Richards and Loomis, 1942; Stocker and Kausch, 1952; Kausch, 1955; Peters, 1957) that water does not move in a root-free soil at a rate adequate to supply roots a number of centimetres way. This is to be expected from physical theory (Philip, 1957a), although it is also to be expected that if root density is high enough, for example, in potted

plants, water could move through a few millimetres of soil at a sufficient rate to maintain adequate rates of supply (Richards and Weaver, 1944; Peters, 1957). Under field conditions, however, root density would normally be inadequate to enable this to occur, and continued root extension would appear to be necessary to maintain significant rates of absorption. This interaction between root density and rate of soil water movement is clearly of primary significance in assessing the relative importance of root growth in determining rate of supply of water to the root, and the influence of transpiration in determining rate of demand is likewise pertinent. Thus, while Weaver and Zink (1946) observed that removal of half the root systems of several grass species had little effect on growth, and Bialoglowski (1936) and Elazari-Volcani (1936) found that with slow rates of transpiration the root surface/leaf surface ratio of citrus could be reduced by about half without effect, many other investigators, particularly those concerned with field crops in drying soils, found that any significant reduction in root surface restricts absorption (Nutman, 1934; Grier; 1940; Leonard, 1944; Parker, 1949).

The fact that rates of flow of unsaturated soil water are very low in soils drier than field capacity emphasizes the importance of root extension for continued absorption. The figures of Kramer and Coile (1940), who computed from data of Dittmer (1937) that extension of the roots of a winter rye plant can provide up to three litres of water a day for absorption, highlight the importance of this feature. The apparent cessation of absorption at the permanent wilting percentage, which is probably related to the cessation of root growth, likewise demonstrates the point. In most cases it appears that the overall limitation to rate of water supply to the root surface will be the progressive effect, during soil water extraction, of the decreasing water potential in the plant-soil system inhibiting further root extension and reducing unsaturated soil water movement. Other factors affecting root growth, such as aeration, temperature and mechanical impedance, can be expected to become major sources of influence only under special circumstances.

ENTRY OF WATER INTO THE ROOT

Although the factors which influence water supply at the root surface are directly associated with the entry of water into the root, in some instances the mode of action differs. It is of value to discuss the factors affecting water entry in relation to zones of differential

absorption at the root epidermis, permeability of the root cortex to water movement and the water potential at the root surface.

THE ABSORBING ZONE OF ROOTS. Examination of roots as absorbing organs has been an aspect of active physiological study for many years. Recent reviews by Kramer (1949, 1956d) provide comprehensive summaries of these investigations, and only the primary aspects will be mentioned here.

In general, it can be stated that the zone of most rapid absorption lies in the region where the xylem is fully mature, between the elongation zone close to the root tip and the suberized zone farther back. In the meristimatic region at the tip, absorption is slow because of the high resistance to water movement offered by the compact cell arrangement (Kramer, 1956e). Absorption is also restricted in the zone of cell elongation because the xylem is not completely differentiated. Maximum intake of water appears to occur in the region where the xylem is fully mature but where the endodermis and epidermis have not become impermeable. This region has been located from 1.5 cm to 20 cm from the root tip, although it is usually found between 5 and 10 cm from the tip (Sierp and Brewig, 1935; Brewig, 1936a, 1937; Hayward and Spurr, 1943; Brouwer, 1953, 1954). The length of the region is very restricted in slow growing roots and appears to increase rapidly with rate of growth (Kramer, 1956d). Beyond this zone of rapid uptake, rate of absorption decreases rapidly as permeability is reduced by increasing suberization and thickening of the walls of the endodermal cells and the suberization or lignification of the epidermal or hypodermal cells (Hayward and Spurr, 1943; Rosene, 1937, 1941; Esau, 1953).

The zone of most rapid absorption is observed to shift during transpiration. This phenomenon has been attributed by Brouwer (1953, 1954) to increased permeability in the older part of the root when turgor is reduced, although other explanations have also been proposed (Mees and Weatherley, 1957b). The significance of transpiration to root permeability is discussed in more detail elsewhere in this paper.

Although most studies have concentrated on zones of most rapid absorption, there is evidence that considerable quantities of water can be absorbed through suberized roots, at least under some conditions. Investigations on citrus trees have shown that, although few unsuberized roots are found on these species during winter, they are far from inactive at this time of year (Chapman and Parker, 1942; Hayward, Blair and Skaling, 1942). Direct studies have confirmed that absorption

occurs through suberized roots (Crider, 1933; Nightingale, 1935; Addoms, 1946), and the fact that such a large proportion of the roots of all terrestrial plants are suberized makes it probable that significant quantities of water pass through such tissue.

The region of most rapid absorption appears to coincide with the normal zone of root hair development. This relationship is to be expected, knowing the importance of root hairs to absorption through their high permeability and their effect in increasing the total root surface (Rosene, 1943, 1954; Kozlowski and Scholtes, 1948; Dittmer, 1949). Estimates of the increase in root surface due to root hairs vary considerably. Some suggest that up to ten times the root surface is developed (Evans, 1938); others that the value is about 1.6 (Dittmer, 1937). With respect to permeability, Rosene (1943, 1954) has found that root hairs are about as permeable to water as the unsuberized epidermis of young onion roots. As is to be expected, their permeability decreases with age (Rosene and Walthall, 1954). Kramer (1956d) comments that root hairs are probably of little influence in total absorption from well watered soil, but whenever total root surface in contact with soil becomes a limiting factor in absorption, presence of root hairs may be of considerable importance.

MOVEMENT OF WATER ACROSS THE CORTEX. There are two main factors concerned with the movement of water across the root cortex: the water potential gradient across the root from the soil to the xylem, and the resistance to water movement caused by root permeability.

The water potential gradient across the root will vary with water potential at the soil-root surface and with fluctuations in the osmotic potential of the xylem sap and the tension developed in the xylem through transpiration. In the absence of transpiration the osmotic potential of the xylem sap alone will presumably determine the water potential at this point. The soil water potential will decrease as soil moisture tension increases (following water extraction) or as the concentration of the soil solution is increased (due to the effect of decreasing soil water or of added solutes). Both these effects will be of greater significance if water uptake by the root is more rapid than water supply to the root, in which case the water potential will decrease more rapidly at the root surface than in the soil mass.

Although the water potential gradient is the driving force causing water movement across the root, the rate of movement is also influenced by the resistance to flow; and as resistance increases, a steeper gradient is needed to maintain the same flux.

Evaluation of the water potential gradient from soil to root has recently aroused some controversy. For a number of years the views of the workers at Riverside (Bernstein and Hayward, 1958), that the soil water potential includes osmotic components, have been prominent. On the other hand, Walter (1955) considered that, since the solutes contributing to the osmotic potential of the soil water are diffusible into the plant, the ideal osmometer concept implicit in the Riverside hypothesis is invalid. Supporting the Walter contention, Philip (1958b) and Bonner (1959) proposed that the effects observed by the Riverside group could be explained, not on the basis of osmotically induced "physiological dryness" but, instead, by the development of a vapour gap which would effectively constitute the plant as an ideal osmometer, or by entry of the diffusible solutes into the plant in toxic quantities. The Riverside group (Bernstein, Gardner and Richards, 1959) disputed the occurrence of a vapour gap, and held that their experimental evidence is logically interpreted by the osmometer concept, a view supported, in general terms, by a number of other authors (see Crafts, Currier and Stocking, 1949; Kramer, 1949; Richards and Wadleigh, 1952). However, experimental data also are available to support Walter's basic premise that the solutes are freely diffusible (Maximov, 1929; Eaton, 1942).

It seems probable that the metabolic processes involved in salt absorption are of importance in this regard (Epstein, 1956a; Gauch, 1957; Robertson, 1958), particularly in view of the fact that respiratory inhibitors normally cause a severe reduction in uptake of both anions and cations (Ordin and Jacobson, 1955). Also, poisoning of the roots and elimination, not suppression, of the active processes has been observed to result in absorption of salts in proportion to their concentration in the external solution (Hoagland and Broyer, 1942).

Recently Scott Russell and Shorrocks (1959), repeating earlier work on ion uptake, have shown that when internal salt status of both the plant and the external medium are low, rate of transfer of ions to plant shoots is independent of the rate of transpiration. However, the combination of high salt status, internally and externally, can cause uptake to vary with rate of transpiration; and concentration of ions in the transpiration stream can be lower than that of the external solution. These results indicate the existence of a barrier which can offer high resistance to the passage of ions across the symplast of the plant roots.

Should such a barrier, also proposed by Bernstein and Hayward

(1958), exist, it seems that the controlling mechanism is located in, or external to, the endodermis, since once ions have entered the free space, their distribution through the rest of the plant would probably proceed rapidly. The possible special importance of the epidermis as an absorbing organ is suggested by the work of Sandstrom (1950) who demonstrated that removal of epidermis of wheat roots resulted in an increase in salt absorption which was wholly passive and proportional to water absorption.

Apart from this movement of water in relation to gradients of water potential, which is primarily passive, active absorption is rapidly inhibited as the water potential decreases below the reference level of pure free water. Most recent evidence indicates that it can proceed only when the soil water potential is not less than about -2.10^6 ergs.gm^{-1} (Eaton, 1943; McDermott, 1945; Hagan, 1949; Army and Kozlowski, 1951). Levitt (1947) calculated that active absorption could possibly maintain a gradient of $1–2.10^6$ ergs.gm^{-1} across the root. This is in close agreement with the values just cited, but as active absorption generally ceases when the external water potential is about -2.10^6 ergs.gm^{-1}, metabolism is presumably retarded and permeability decreased to such an extent by reduced hydration that no net transfer of water occurs.

In general, reduced hydration results in reduced permeability, and most studies which have related permeability to the concentration of external solution have obtained this result (Levitt, Scarth and Gibbs, 1936; Aykin, 1946). On the other hand, Bogen (1940, 1941) and Myers (1951) observed that plasmolysed cells are more permeable than those unplasmolysed. It seems probable that any marked and prolonged reduction in turgor, leading to disruption of normal metabolism, has a direct and severe inhibitory effect on permeability and hence on absorption. In this regard Mees and Weatherley (1957a, 1957b) noticed a decline in root permeability after several hours of induced hydrostatic pressure, even though the initial effect was to cause a marked increase in permeability. Kramer (1950) concluded that prolonged water stress causes an immediate decrease in permeability due to the increased resistance of dehydrated cell membranes and a delayed effect caused by changes in the structure of the protoplasm. It is clear that the tensions across the root resulting from the influence of transpiration must be interpreted differently to the effect of a general decrease in the water potential of the root tissue associated with decreasing soil water potential.

The permeability of the root to water is markedly affected by the metabolic condition of the root tissue and hence by factors which influence respiration, the energy source for metabolism. This is well illustrated by the inhibitory effect on absorption caused by respiratory inhibitors such as azide or KCN (van Overbeek, 1942; Rosene, 1944, 1947; Mees and Weatherley, 1957b). Under natural conditions the most important factors affecting permeability appear to be hydration, temperature and aeration. Active absorption, being wholly dependent on metabolism, is more severely inhibited than passive absorption when these factors are adverse.

The primary effect of low temperature on absorption appears to be to decrease the permeability of the tissue, directly through an effect on membrane permeability, and indirectly through increased viscosity of the protoplasm and of water (Kramer, 1949, 1956c). As much of the resistance to movement of water decreases if the roots are killed, the former factor is probably of greater importance.

The effect of low temperature on reducing absorption varies considerably with different species, plants native to warm environments being more affected than those from cold climates (Brown, 1939; Schroeder, 1939; Cameron, 1941; Kramer, 1942; Kozlowski, 1943). The effect of prolonged low temperature is more complicated than that of sudden chilling as conditioning to low temperature can result in an increase in permeability and hence in absorption rate (Levitt and Scarth, 1936; Levitt, 1941). High temperatures also influences absorption (Haas, 1936; Bialoglowski, 1936). As active absorption is more sensitive to high temperature than passive absorption (Kramer, 1940a), it would appear that the high temperature effect is also due to disruption of normal metabolism. Ellis and Swaney (1947) considered that inadequate oxygen supply is important at high temperatures, and Hagan (1952b) has suggested that direct heat injury to fine roots and root hairs could be important.

The effect of temperature on permeability is closely linked with that of aeration, since the oxygen requirements for respiration increase rapidly with temperature. Under field conditions marked differences in response to lack of aeration have been observed, some investigators finding that flooding markedly reduces absorption, others that it is little affected (Parker, 1950; Hunt, 1951; Kramer, 1951). Differences between species are also important, water plants being relatively unaffected by lack of aeration and many land plants showing extreme tolerance to flooding (Kramer, 1949; Russell, 1952). As might be ex-

pected, plants are usually less affected if dormant, and the degree of injury becomes more pronounced with increasing temperature (Kramer and Jackson, 1954).

Lack of aeration is probably of influence through the combined effects of low oxygen and high CO_2 concentrations, although the two factors have somewhat different modes of action. The effects of high CO_2 appear to be very rapid (Kramer, 1940b, 1945; Hoagland and Broyer, 1942; Chang and Loomis, 1945; Hagan, 1950; Kramer and Jackson, 1954) and are probably caused by the direct toxic effect of high CO_2 on permeability (Russell, 1952; Kramer, 1956c). Oxygen deficiency, on the other hand, is slower acting and may take hours or even days to become apparent (Whitney, 1942; Hoagland and Broyer, 1942; Rosene, 1950; Rosene and Bartlett, 1950; Mees and Weatherley, 1957b). This delay is closely related to the degree of oxygen deficiency, total absence of oxygen in small containers usually inhibiting absorption in several hours (Mees and Weatherley, 1957b). Kramer (1949) suggested that the longer delay normally encountered may be due to the fact that some oxygen is present in the tissues and that some may diffuse down from the shoots, thus deferring the onset of oxygen shortage and inhibition of aerobic respiration. He also suggested that if one of the primary effects of low oxygen is a build up of toxic end products of anaerobic respiration, some time may elapse before this accumulation becomes important. Probably both these explanations are valid.

Some investigators have noted an increase in absorption rate following the initial decrease caused by lack of aeration. This has been generally attributed to the breakdown of root tissue following permanent damage (Russell, 1952). The results of Mees and Weatherley (1957b) support this contention, and they concluded that, as the effect is not reversed by subsequent aeration, it is due to death of the cells. In general, lack of aeration appears to act through reduced oxygen supply, not through excess CO_2, particularly if it proceeds for several days. Whitney (1942) observed that even high CO_2 concentrations are of little consequence if the oxygen concentration is adequate. Russell (1952) concluded that, under field conditions, high CO_2 is likely to be of minor significance unless conditions also favour very low oxygen levels.

Most of the factors affecting movement of water through the root have been considered here with special reference to their immediate effects on absorption. It must be recognised that the indirect or longer

term effects are also of significance. In particular the effects of adverse conditions—low water potential, lack of aeration, low or high temperature—on root growth and development are important in this regard. Resultant inhibition of root growth, increased suberization and death of root hairs all reduce absorption over a period of a few days. In addition, the conditioning of plants to, say, low temperature may result in changes in permeability which can affect rates of absorption.

The effect of the various factors on controlling absorption are varied, but all significantly affect absorption under certain conditions. Decreasing soil water potential inhibits active absorption at levels below about -2.10^6 ergs.gm^{-1} (Eaton, 1941, 1943; Kramer, 1941; McDermott, 1945; Jannti, 1953; Jannti and Kramer, 1956) and progressively restricts passive absorption as it decreases over a wider range (Chung, 1935; Martin, 1940; Eaton, 1942; Hayward and Spurr, 1944; Army and Kozlowski, 1951; Corey and Blake, 1953; Bloodworth, Page and Cowley, 1956; Slatyer, 1956b, 1957a). Low temperature also severely inhibits absorption and transpiration, and frequently results in marked wilting of leaves (Döring, 1935; Brown, 1939; Schroeder, 1939; Kramer, 1942). Deficient aeration can reduce transpiration by more than 50 per cent, with associated wilting (Kramer, 1938, 1940b, 1945; Whitney, 1942; Parker, 1950). Although these factors, acting through reduced root permeability or decreased soil-xylem water potential gradients, frequently appear to be direct in action, the van den Honert hypothesis raises the possibility that they may be indirect, acting primarily through reducing the water potential in the leaves and hence causing stomatal closure and reduced transpiration. This subject will be discussed in detail below.

PASSAGE OF WATER IN CONDUCTING ELEMENTS

Aspects of water movement in the conducting elements have already been discussed. In these paragraphs the primary interest is to evaluate the resistance to movement in the conducting elements and understand the factors influencing it. The recent review by Greenidge (1957) may again be cited for a more detailed treatment of the general aspects of sap ascent.

Experimental work has proved conclusively that rapid longitudinal movement of water in plants can take place through the non-living elements of the xylem (Crafts, Currier and Stocking, 1949). This in itself is evidence of low resistance to movement; and the phenomena

of guttation and root pressure, which are manifestations of active absorption processes, likewise demonstrate that little resistance occurs.

It is to be expected that such resistance as does occur may be attributed to breaks in the cohesive columns, to the physical effect of the height of the plant or to the conductive capacity of the elements. The occurrence of breaks in the elements appears to have surprisingly little effect on water movement, so is presumably a source of minor resistance in the system. Even drastic treatments, such as overlapping horizontal cuts in the stem (Elazari-Volcani, 1936; Preston, 1952; Greenidge, 1955a, 1955b, 1958), only slightly retard movement, and introduction of gas into the elements does not prevent large vine and liana stems from regaining their previous absorption rates when water supply is re-established (Scholander, Love and Kanwisher, 1955; Scholander, Ruud and Leivestad, 1957). The hypothesis that water moves freely around overlapping cuts in the bole has been substantiated by the recent demonstration by Postlethwait and Rogers (1958).

Although the evidence suggests that little resistance occurs, a hydrostatic gradient is to be expected through a transpiring plant due to the length of the stem and its gravitational head. Thus with increasing height the tension in the system needed to maintain a constant rate of movement can be expected to increase. Most studies reveal that only a small gradient occurs with height, usually 0.2–0.5 atm.metre^{-1} (Renner, 1911, 1912; Dixon, 1914; Eaton, 1941; Stocking, 1945), but Arcichovskij and Ossipov (1931) observed a gradient of as much as 44 atm.metre^{-1} in a desert shrub. Although the experimental technique used by these authors was subject to some errors, this general order of resistance presumably occurred and could possibly have been a result of the general degree of desiccation of the plant, resulting in almost complete absence of water columns.

The effect of adverse environmental conditions on resistance to water movement through the stem has not been extensively investigated, although Handley (1939) investigated the influence of low temperature on upward water movement in two tree species. He found little resistance to movement until the temperature was reduced below 2°C., when marked reduction of water supply to the leaves occurred and wilting ensued. More recently Johnston (1959) found no reduction in stem flow of *Pinus radiata* until freezing of the stem occurred, and he considers that Handley's data should also be re-interpreted in this manner.

Resistance to flow of water in the xylem elements increases rapidly

when very small diameters are encountered, as, for example, in grass roots. Emerson (1954) and Wind (1955a, 1955b) studied this subject in some detail, and the latter author concluded that the resistance to flow in metaxylem elements of radius less than 20 μ is so great that it exceeds the resistance to unsaturated water movement through the soil. On this basis, Wind explains the absence of grass roots below about 20 cm in moist soils. Although these conclusions are open to question, particularly because his soil water data apply to capillary rise of water in moist soils and not to movement in dry soils, the significant resistance to water movement in small roots must be recognized. It is probable, however, that for most herbaceous plants the xylem and metaxylem elements are of adequate radius to permit movement at low resistances.

MOVEMENT OF WATER THROUGH AND OUT OF LEAVES

The primary factor causing water movement through the leaf, as through the plant, is transpiration.

Transpiration can, to a considerable extent, be regarded as a purely passive process, the rate of which is determined primarily by the vapour pressure gradient between the leaf and the outside air and by the various resistances to water movement in the transpiration path. Ordinary evaporation of water from porous materials is also affected by these considerations so that in general the effect of atmospheric conditions on transpiration will parallel their effect on evaporation. A valuable recent review on this subject is available (Milthorpe, 1959).

The important physiological factors influencing transpiration are those which cause resistance in the transpiration path and which directly or indirectly affect the vapour pressure gradient to the outer air. These can be arranged in two groups: those which cause resistance to movement in the gaseous phase and those which cause resistance through the cell walls.

Van den Honert (1948) considered that, for a plant freely supplied with soil water, under normal atmospheric conditions, the resistance to transpiration in the gaseous phase is of the order of 20 times the resistance through the rest of the plant. At first sight this may seem improbable, but the main source of resistance appears to be in the air layer adjacent to the evaporating surfaces of the cells, in which a steep diffusion gradient is found leading to the freely circulating air beyond the leaf surface. Van den Honert considered that, because of

the magnitude of this factor, resistance to movement through open stomates is only about ten per cent of the gaseous phase as a whole. He subsequently concluded that the effect of stomatal closure on transpiration, and hence on absorption, cannot be expected to exert control until its resistance balances that of the gaseous phase itself. Van den Honert considered that such an effect arises only at small stomatal apertures.

The role of stomatal closure in controlling transpiration has been difficult to determine because of the fact that stomatal reactions are frequently associated with decreasing water content. Most recent studies tend to support van den Honert's hypothesis and show that stomatal control is relatively ineffective until the aperture is reduced by about half, beyond which point very effective control is exercised (Mendel, 1944; Brueckner, 1945; Crafts, Currier and Stocking, 1949; Bange, 1953; Hygen, 1953; Milthorpe and Spencer, 1957; Williams and Amer, 1957), although Kuipen and Bierhuizen (1958) have demonstrated effective stomatal regulation over a wide range of light intensities. Most of these studies have been conducted in the laboratory where resistance to diffusion outside the leaf can be a major factor in the total diffusion resistance in the gaseous phase. Under natural conditions, mobility of the leaf and turbulent eddy diffusion reduce the external resistance appreciably, and Philip (personal communication) considers that even the resistance of open stomates could be a significant factor.

In general, with decreasing water content, a constant rate phase of transpiration first occurs, during which little stomatal control is exercised. This is followed by a falling rate phase, during which stomatal closure progressively inhibits transpiration, and subsequently by a cuticlar phase when the stomates are completely closed. Superimposed on this picture is the effect of decreasing water content, in which loss of water follows the normal behaviour to be expected from water evaporating from porous materials (Gilliland, 1938). This also shows an initial constant rate phase followed by a sudden change to a falling rate phase in which the rate drops rapidly with decreasing water content. Although Gregory, Milthorpe, Pearce and Spencer (1950a, 1950b) and Milthorpe and Spencer (1957) maintained that leaf water content *per se* is of no consequence in controlling water loss, it is difficult to separate the direct and indirect effects of this factor.

The critical water content at which the falling rate phase commences is of obvious importance with respect to transpiration. Gregory

et al (1950b) found that with the experimental material and conditions they used, it is about 90 per cent of the turgid water content. With more rapid transpiration it could be expected to be higher, and a wide variation in different types of plant and plant tissues could also be expected. Even so, a value of 90 per cent probably represents a water potential of only -5–10.10^6 ergs.gm^{-1}, a value commonly attained in transpiring plants under natural conditions, and the possible significance of low leaf water content must accordingly be recognized.

The mode of action of this factor on reducing transpiration is obscure. Although it is clear that any retreat of the evaporating surface, involving a lengthening of the diffusion path, will tend to increase the resistance in the gaseous phase, it is also possible that partial drying of the cell walls may be of influence. A reduction of water content in the leaf will be associated with a partial drying of the mesophyll cell walls, and Preston (1954) considers that this increases the resistance of the walls to water movement. Supporting evidence for this view is found in the work of Klemm (1956) who noted that evaporation from exposed cell surfaces, under conditions of low humidity, was much less than that from moist filter paper or multiperforate septa. In addition, the work of Boon-Long (1941) showed that any decrease in the water potential of the mesophyll cells is associated with greatly decreased cell wall permeability, and Meidner's (1955) studies demonstrate a marked increase in mesophyll resistance with decreasing water content.

Any significant increase in resistance to water movement through cell walls may be of importance in controlling transpiration if it is adequate to result in the difference in water potential across the wall being an appreciable fraction of the potential difference across the gaseous phase. Evidence of resistances of this magnitude is lacking at the present time (Milthorpe, 1959), although data that the external mesophyll cell walls are hydrophobic (Lewis, 1945, 1948) indicate that the water potential of the air adjacent to the walls could be appreciably lower than that of the cell. This effect could be expected to be most pronounced in xerophytic and sclerophytic species where cutinization of the walls may occur. Should cutinization be extensive, cell wall resistance in the sub-stomatal cavities could conceivably approach that of the leaf cuticle. This seems improbable for most cultivated plants and indeed for any physiologically active species, since the concommittant reduction in CO_2 exchange would severely limit photosynthesis and growth.

Regardless of the mode of action, it is apparent that in several cases marked changes in transpiration rate have been observed in the absence of measurable stomatal reactions (Went, 1944; Oppenheimer, 1951). It therefore appears that, while stomatal explanations of transpiration regulation are usualy adequate to explain observed phenomena, other factors may also be operative, and these will possibly be associated with phenomena such as those just discussed.

RELATIVE IMPORTANCE OF FACTORS AFFECTING ABSORPTION

If water transport through plants can be regarded as a catenary process, rate of transport dm/dt, through the whole of the soil-plant-atmosphere system, under isothermal and steady state conditions, can be expressed in a form analagous to that of an Ohm's law equation, so that:—

$$\frac{dm}{dt} = \frac{PD_s}{R_s} = \frac{PD_r}{R_r} = \frac{PD_x}{R_x} = \frac{PD_l}{R_l} = \frac{PD_g}{R_g}$$

where R_s, R_r, R_x, R_l, R_g are the resistances in soil, root, xylem, leaf cells and gaseous phase, respectively, and the PD symbols represent the reduction in water potential across each of the appropriate resistances. It must be appreciated that the potential difference in the gaseous phase is in reality the vapour pressure gradient from the evaporating surface within the leaf to the external air. Because of the non-linearity of the water potential/vapour pressure relationship, the magnitude of the resistance in this phase is not usually as great as may appear. However, this does not affect the general validity of this analysis, since under normal conditions the magnitude is the same.

Although, as shown in this expression, each of the partial processes in the system proceeds at the same rate, the general rule regarding catenary processes applies—that the limiting partial process (in this case the partial process encountering the greatest resistance) controls, for practical purposes, the velocity of the whole. Furthermore, in an Ohm's law equation, the resistance in any one of the partial processes is directly proportional to the potential difference, in this case the reduction in water potential. From these two generalisations the relative magnitude of the various resistances in the path of water movement through the soil-plant-atmosphere system can be evaluated and the primary source of control identified.

The water potential in the soil adjacent to the roots normally fluc-

tuates from near zero to about -30.10^6 ergs.gm^{-1}, whereas the water potential in the atmosphere under normal conditions (air temperature 15-20°C, relative humidity 50 per cent) is equivalent to -800-$1{,}000.10^6$ ergs.gm^{-1}. The water potential in the leaf cells normally fluctuates over a range similar to that of the soil, and values below -50.10^6 ergs.gm^{-1} are unusual. From these figures it can readily be seen that the total reduction of water potential from soil to leaf cells seldom exceeds 50.10^6 ergs.gm^{-1}, while the reduction of water potential in the gaseous phase from leaf cells to free atmosphere may be 750-950.10^6 ergs.gm^{-1}. Therefore, the conclusion must be drawn that the resistance in the gaseous phase far exceeds the resistance in the rest of the plant and that, as a consequence, control of water transport is located at this point.

In essence, these are the interpretations and conclusions of Gradmann (1928) and van den Honert (1948), and they appear to be soundly based and logically derived (Edlefsen, 1941; Philip, 1957a). Consequently the question must arise as to the mechanism by which absorption can be controlled elsewhere than in the gaseous phase in the leaf, since, before any control can be exercised directly from another part of the system, the reduction of water potential across that zone must represent a significant fraction of the reduction of water potential across the gaseous phase.

It can be appreciated that, if air temperature is low and humidity high, such a phenomenon may occur. In winter, particularly, it seems possible that resistance elsewhere than in the leaf could be of major importance. Under atmospheric conditions favourable to plant growth, however, such as those defined above, it is difficult to envisage any resistance in the plant of adequate magnitude to be of direct influence because there does not appear to be any part of the plant within which such resistances could arise. The highest water potential values measured or estimated in plants have been about -200.10^6 ergs.gm^{-1} (MacDougal, 1926; Arcichovskij and Ossipov, 1931; Stone, Went and Young, 1950), and even the direct effect of such values as these, under the atmospheric conditions given above, would be to reduce water transport by only about one quarter.

Since the experimental data reported previously in this paper have shown that low soil temperature, inadequate aeration and low water potential can reduce absorption and transport virtually to zero, it seems probable that the mechanism by which such factors normally

operate is not through their direct effect on resistance in the root but through their indirect effect on resistance in the gaseous phase in the leaf, through reduced leaf water content and stomatal closure.

As van den Honert (1948) has pointed out, the gaseous phase is the logical place for regulation of water transport, since regulation elsewhere could result only in rapid dehydration of the portion of the plant between the zone of regulation and the leaf surface. Thus the direct effect of increased root resistance on transport appears to be due to its effect on increasing the water potential difference across the root. Even if this results in only a small reduction of water potential in the rest of the plant, it causes a significant reduction in leaf turgor and probable stomatal closure. In this way the resistance in the gaseous phase is increased significantly, and rate of transpiration and of transport generally is reduced.

Experimental data bearing on this point support this explanation, the immediate effect of any of the factors known to reduce markedly absorption being to cause pronounced loss of leaf turgor and onset of wilting. It is apparent that in order for the maximum reduction in absorption to occur, complete stomatal closure would have to be achieved. Regardless of plant species, it is probable that stomatal closure is complete when the leaves are severely wilted and the water potential in the leaves has dropped to the stage at which there is zero turgor pressure in the active tissue. In most sun plants this represents a water potential of about -15.10^6 ergs.gm^{-1} (Slatyer, 1957b). There are very few quantitative data on the effect of decreased root permeability on the water potential in the leaves, but the fact that severe wilting can follow exposure to low temperature and inadequate aeration or introduction of metabolic inhibitors is evidence that the drop in water potential across the root, in such cases, is of this magnitude. The data of Long (1943) provide evidence of the decreased root permeability which follows a decrease in the osmotic potential of culture solutions. Long observed that plants in substrates of $-0.7.10^6$ ergs.gm^{-1} had a water deficit in their leaves of 15 per cent; in solutions of $-4.8.10^6$ ergs.gm^{-1} the water deficit was 25-30 per cent. Approximate plant water potential values for these deficits (Weatherley and Slatyer, 1957) could be -10 and -20.10^6 ergs-gm^{-1}, respectively, so that the reduction in water potential across the root in the former case could have been 9.10^6 ergs.gm^{-1}, in the latter 15.10^6 ergs.gm^{-1}. It can be appreciated that a water potential of -20.10^6 ergs.gm^{-1} would almost

certainly be associated with severe wilting, complete stomatal closure and reduced water transport. A reduction in transport of 88 per cent was in fact observed in an experiment with substrates of similar concentration conducted by Hayward and Spurr (1943).

The efficiency of stomatal regulation of water transport varies considerably from species to species and with different environmental conditions. If continued water loss occurs in the form of cuticular transpiration, even when the stomates are completely closed, there is a danger of continued dehydration and possible death of the plant. This can occur if the rate of cuticular transpiration is rapid enough and root resistance high enough to result in a considerable reduction of water potential across the root and a resultingly low water potential in the rest of the plant.

It is probable that such a phenomenon frequently occurs when plants die or become extremely desiccated in drying soils. Unless transpiration can be reduced almost to zero, the resistance across the soil-root surface zone in drying soils increases very rapidly, and a steep enough water potential gradient across this zone cannot be developed without severe dehydration and probable death of the top of the plant. Kramer (1942, 1956c) considers that "winter injury" is brought about by a mechanism such as this, since in cold soils (particularly if frozen) considerable resistance to water movement in the soil-root surface and root surface-xylem zones develops; and if a period of warm sunny weather occurs, dehydration of the top of the plant takes place.

From this analysis the relative importance of the various factors affecting absorption can be seen to depend very largely on the "filtration" resistance which they cause, for this determines the water potential further along the path of water movement and ultimately affects the resistance in the gaseous phase. On this basis the only segment of the transpiration path in which major sources of resistance appear likely to occur are in the soil-root surface and root surface-xylem regions. In the stem it appears that little resistance to water movement occurs except in tall trees or in cases where the stem is at freezing temperatures. The latter effect appears unlikely to arise in practice, as under such temperature conditions it is highly probable that transpiration would be near zero or that, in such cases, resistance in soil and root would be even higher than in the stem. Resistance in the leaf also appears to be low in most cases, even though the

indirect evidence reported previously suggests that under some conditions it could be of significance.

In the soil-root surface zone, low resistance to movement exists only while the soil water is at very low tension or while root extension continues. As soon as drying commences and root extension ceases, very high resistance to movement develops. In the root surface-xylem zone, resistance is low also in the absence of adverse environmental conditions and other factors adversely affecting metabolism, and as long as root surface area is adequate. The onset of adverse conditions has a direct effect on metabolism and an indirect effect on the absorbing surface of the roots, both these features contributing to a rapid increase in resistance to water movement across the root. Although low temperature or deficient aeration can reduce transpiration almost to zero, the most important overall factor affecting water transport appears to be low soil water potential at the root surface. This can be of influence in three ways. It has a direct effect on the water potential in the plant, since, in order to maintain a gradient favouring absorption, the water potential in the plant must always be lower than that in the soil. It has an indirect effect on root resistance through the effect of hydration on permeability and suberization. Finally it has a direct effect on resistance to water movement through the soil-root surface zone, which influences the water potential gradient needed to maintain transport across the zone. It can be appreciated that resistance to water movement through the soil can become of sufficient magnitude to limit directly water transport. However, this rarely occurs in living plants, since plants die of dehydration before the resistance reaches a high enough level.

To conclude this portion of the paper it may be stated that examination of water absorption by plants in terms of the van den Honert hypothesis leads to the conclusion that most of the factors which result in decreased absorption act indirectly through the effect of reduced water potential on decreasing transpiration by the leaf instead of directly in the region in which they occur. The van den Honert hypothesis appears sound, although its application may need more attention. In this regard a fuller realisation is needed of all the sources of resistance and their potential orders of magnitude. The latter factors may be of special significance if, as Preston (1954) has suggested, resistance in the leaf mesophyll cell walls be of an order similar to that of the gaseous phase. If such resistance were to develop, direct

control of absorption at the root would be much more probable. At present, however, the evidence for such a source of resistance is inadequate.

ABSORPTION OF WATER BY LEAVES AND OTHER AERIAL ORGANS

Studies of the absorption of water and of water vapour by the above ground parts of plants have been conducted for many years, yet present knowledge of the extent and significance of such absorption is inadequate, and considerable controversy still exists concerning some aspects of the phenomenon.

The present discussion is concerned with the processes involved in the absorption of water in this manner, the extent to which absorption occurs and the significance it has to the water economy of the plant. Two recent reviews are available for more extensive coverages of this literature (Gessner, 1956; Stone, 1957a).

PROBABLE MECHANISMS INVOLVED

The factors associated with the entry of atmospheric water into, and its passage through, a plant appear to be those which determine the surface characteristics of the leaves, the resistance to movement within the plant and the water potential gradient across the atmosphere-plant-soil system. As with normal absorption, rate of water uptake will depend on the gradient developed and the resistance to flow.

ENTRY OF WATER INTO THE LEAF. Wettability of the cuticle appears to be an important prerequisite for absorption. The ability of a liquid to wet a surface is a function of its contact angle on the surface; in turn this depends on the surface tension of the liquid and the nature of the surface. As would be expected from the work of Wetzel (1924) and Marloth (1926) who showed that pubescence and the presence of hydrophobic surface agents reduced water uptake, Fogg (1947) noted that marked differences exist in the contact angles of water on several species, and that the contact angle is influenced by age of leaf and water content. Fogg attributed these differences primarily to variations in surface conformation, pubescence and the composition of the cuticle. As contact angle is reduced by the addition of wetting agents (Ebeling, 1939), it could be expected that the addition of such materials would materially improve absorption at least on some types of

leaves. In this connection the addition of detergents to foliar sprays (Guest and Chapman, 1949; Cook and Boynton, 1952) has been found to materially increase nutrient uptake, and, although no studies dealing with water absorption have been made so far as the author is aware, a similar result could be expected in such cases.

Although a considerable amount of data is now available on foliar applications of nutrient sprays (Boynton, 1954), the fact that nutrient uptake takes place even when the leaf appears to be dry is evidence that other mechanisms are operative besides those associated with water absorption. Even so, some of this work is of considerable value in understanding the path of entry of water into the leaf. Thus, Roberts, Southwick and Palmiter (1948) found that in apple leaves the cutin of the epidermis was in discontinuous lamellae parallel to the outer epidermal wall. Interspersed with the cutin lamellae, pectinaceous substances were found to occur in intermittent layers in the outer epidermal walls and appeared to form a continuous path from the layers in the cuticle through the anticlinal walls of the epidermal cells to the cell walls of the vein extensions and bundle sheaths surrounding the larger veins of the leaves. These authors considered that this could provide a pathway for water movement from the cuticle to the living cells surrounding the vascular tissues. A separate study by Palmiter, Roberts and Southwick (1946) demonstrated that solutes did move along this pathway; and Steubing (1949), using flourescent dyes, observed water movement through the walls in a similar manner. On more recent considerations it seems probable that most of the free space would be available for the rapid transfer of both water and solutes once they have crossed the epidermis (Epstein, 1956b; Kramer, 1957).

The relative importance of stomates as the points of initial water entry is still obscure, although it is clear that absorption of nutrients and insecticidal oils frequently occurs in this manner (Knight, Chamberlain and Samuels, 1929; Ginsburg, 1930; Kelley, 1930; Rohrbaugh, 1934; Cook and Boynton, 1952). Crafts (1933), however, regarded absorption through stomates as negligible; and Turell (1947), arguing on the basis of contact angle and capillarity, supported this contention, although he considered that addition of wetting agents could result in some absorption. Gessner (1956) thought that in most cases water penetration occurs directly through the cuticle, and the studies of Zamfirescu (1931) and Meidner (1954) raise the possible impor-

tance of specialised epidermal cells in water uptake. The latter author, in particular, noted that most of the water absorbed by *Chaetacme aristata* leaves occurs through specialised cells in the epidermis. He also noted that absorption by the upper leaf surface, where most of the cells are located, is much greater than on the under surface where stomatal frequency could be expected to be highest, and this observation may also provide some evidence as to the significance of stomatal absorption.

In general, and with the present state of knowledge, it appears that most of the water absorbed by leaves is through the cuticle. If this is so, a marked increase in cuticle permeability must occur on wetting in order to explain the paradox of high cuticle resistance to water transport during transpiration. The evidence for any conclusive remarks is inadequate and this question must still be regarded as open. Little work has as yet been conducted on water entry through organs other than the leaf.

Rate of absorption is markedly affected by the potential gradient from air to leaf (Krause, 1935; Slatyer, 1956a), and it is to be expected that initial rates of uptake would be greatest, the rate decreasing progressively as the leaves regain turgor and the gradient is reduced. Eisenzopf (1952), however, observed a peak rate of absorption after 90 minutes immersion in water, following which rate of uptake decreased fairly rapidly. It seems probable that the increased rate for the first 90 minutes was caused by a progressive increase in cuticle permeability as a result of increased cuticle hydration. The decreased absorption after this time was attributed by Eisenzopf (1952) to decreased permeability of the cuticle, although a more probable explanation is that the water potential gradient had been progressively reduced.

TRANSFER OF WATER THROUGH THE PLANT. As has been mentioned previously in this paper, movement of water through the plant may be validly interpreted by regarding the soil-plant-atmosphere system as a thermodynamic continuum. Such considerations apply equally well to the movement and redistribution of water which is absorbed through the leaves. However, experiments conducted on this subject have to date given conflicting results, and it appears that in many cases resistance to movement is such that the gradients established are inadequate to cause movement.

The most frequently cited experiments concerning negative trans-

port through the whole atmosphere-plant-soil system are those of Breazeale, McGeorge and Breazeale (1950, 1951) and Breazeale and McGeorge (1953a, 1953b). In addition to these studies, Haines (1952, 1953), Stone, Shachori and Stanley (1956) and Slatyer (1956a) observed negative transport when roots were placed in empty flasks and a steep water potential gradient had been established. Although these experiments appear to provide a sound physical explanation for water absorption and transfer, several other experiments conducted along similar lines have not demonstrated transport. Thus with plants rooted in soil, even when the soil was as dry as the permanent wilting percentage and an adequate gradient should have been established, no accumulation of water in the soil has been observed by some investigators, even though the leaves of the plants under study regained turgor (Hohn, 1954; Janes, 1954; Stone, Shachori and Stanley, 1956). This is somewhat surprising in view of the fact that Hagan (1949) and Bormann (1957) demonstrated sufficient cross transfer of water through inter-twined root systems to maintain growth of plants with no other water source. Hagan observed rapid negative transport when plants in dry soil had been detopped and the stumps connected to potometers.

It seems probable that several factors are of influence in causing these conflicting results. In the first place absorption of water by leaves, although occurring in most species if a favourable gradient exists, generally appears to be very slow because of the resistance of the cuticle to water entry, and the quantities of water absorbed may be quite small. Transfer of this water to other branches and to the roots has also been observed (Dixon, 1924; Brierley, 1934, 1936). However, because of the small amounts of water absorbed by the leaves, and because the rate of transfer also appears to be fairly slow, the amount of water transferred is in some cases not sufficient to enable recovery of turgor by other parts of the plant. Because such small amounts of water are involved, it seems probable that in many instances the lack of accumulation of water in the root medium may be due to inadequate rate of water intake by the leaves.

This could be expected to be more noticeable when the roots are in soil than when they are in air, since the water potential gradient from root to soil will not normally be as great as that from root to air, particularly if in the latter case the temperature of the flask con-

taining the roots is permitted to fluctuate appreciably. The fact that Hagan (1949) observed rapid negative transport into soil when rate of supply was non-limiting supports this contention; and the data of Hohn (1954), which showed that negative transport into the vapour surrounding the roots does not occur until the relative humidity falls to 85 per cent, is evidence of the gradient needed, in some cases, to cause water movement through the system. Stone, Shachori and Stanley (1956) also noted that the relative humidity in the flask containing roots falls to about 85 per cent on warm days. This is equivalent to a water potential of about -200.10^6 ergs.gm^{-1} at the temperature employed, and is considerably in excess of the probable root-soil gradients which develop with plants rooted in soil. It must again be recalled that in dealing with vapour transport it is the vapour pressure gradient, not the water potential gradient, which determines the rate of water movement. As in transpiration, however, use of water potential considerations does not appear to affect the validity of the conclusions which are drawn in this discussion.

In most instances the chief source of resistance to negative water movement appears to be in the leaves, and rate of movement across the cuticle may be the limiting process (Slatyer, 1956a). Although Hagan's (1949) data indirectly support this hypothesis, the fact that other studies have failed to demonstrate negative transport suggests that significant resistance to water movement may also occur at the root surface. Slatyer (1957a) suggested that the death of small roots and root hairs in dry soil, together with rapid suberization, could markedly reduce the root surface available for transfer and hence increase the resistance to movement across this zone. If, as a result of soil and root shrinkage, a vapour gap were to develop between root and soil, a further important resistance could arise. If such phenomena occur, the failure in some experiments to observe negative transport, while at the same time recovery of turgor by the plant tops is noted, could be largely explained. An alternative explanation for the lack of transport into dry soils might be that if some exudation occurred into the soil layer directly adjacent to the root surface, the soil water potential in this layer would be effectively raised to a value approximating that at field capacity, and the gradient from atmosphere to soil would be eliminated. Unless the soil became completely saturated, water movement from this zone into the soil mass would not be expected because of the very slow rates of unsaturated water movement.

As a result the water in this narrow zone would be at a water potential close to field capacity, but the bulk of the soil mass would remain dry.

Because most studies involving roots in air have demonstrated negative transport (as long as the temperature of the flask containing the roots has been permitted to fluctuate) and most studies with roots in soil have not given this result, it seems that the main reason for lack of transport into soil is lack of an adequate gradient. In addition, in some cases when plants were rooted in soil, not only has there been no accumulation of water in the soil, but, on the contrary, continued transpiration has occurred and resulted in further soil water depletion (Hohn, 1954; Janes, 1954). This throws some doubt on the efficacy of the experimental methods in establishing water vapour saturation around the aerial parts of a plant, or in preventing leaf temperature from rising significantly above air temperature. Such contol is not as important in the case of plants rooted in air because of the much lower water potentials around the roots.

With roots in air, transpiration is greatly restricted by the absence of a water source around the roots. It seems possible that in some experiments with plants rooted in soil, negative transport may occur to a limited extent at night, but transpiration during the day results in a net loss over 24 hours. Any negative transport which occurs with plants in air results in accumulation of water in the bottom of the flask, and only a very small proportion of this water is subsequently available for transpiration.

While most evidence points to transport being subject to controls which are predominantly physical in origin, some experiments are not directly explicable on this basis. Breazeale, McGeorge and Breazeale (1951) observed transport into culture solutions of low osmotic concentration and in soils wetter than field capacity. To explain these results, Breazeale and McGeorge (1953a) proposed that an exudation pressure is developed, presumably in the leaves or roots, during transport. Haines (1952, 1953), in a repetitive experiment, was unable to find any evidence of a non-physical system, and Wiersma and Veihmeyer (1954) found that the exudation pressure measured by Breazeale and McGeorge could be explained solely on the basis of gases produced by decomposition of the plant roots. In the absence of further experimentation, a satisfactory explanation of the original results of Breazeale, McGeorge and Breazeale (1951) and of those workers

who have observed continued transport of water into soil at field capacity must be deferred.

SIGNIFICANCE OF ABSORPTION IN NATURE

It seems that absorption of water by the tops of plants can be of possible influence to the water economy of the plant, either directly by increasing the amount of water in the plant, or indirectly by reducing transpiration. Both these factors tend to reduce the absorption lag behind transpiration, increase turgor and promote plant growth. Under natural conditions such phenomena could possibly occur as a result of wetting of the leaves by rain, dew or sprinkler irrigation or by the presence of very humid air. Greatest interest is centered around the possible utilisation of dew because the presence of rain or irrigation implies water abundance and minimises the importance of absorption through the aerial organs; also because in arid regions the amount of dew can be quite an appreciable proportion of the total precipitation (Duvdevani, 1953).

There are two main reasons why dew, which never comprises more than a small proportion of the water requirements of a normal plant, could be an important water source. These are that it occurs at night when transpiration is negligible and when the effect of increased leaf turgor is likely to have the greatest effect on leaf expansion, and that it frequently remains on the leaves for several hours after sunrise, thus reducing transpiration while permitting photosynthesis.

Most experiments designed to evaluate the significance of dew have shown that the beneficial effects observed could be attributed solely to the presence of surface water on the leaves and that it is not necessary to consider the possibility of absorbed water. Thus Walter (1936, 1951) concluded that the primary effect of dew in the South West African deserts is in reducing transpiration, while the leaves remain wet in the mornings following dew nights. Duvdevani (1953) reported that plants exposed to dew develop greater length and number of branches and greater leaf area than those not exposed. Although Duvdevani considered that this is due in large measure to the effect of dew absorption by the plants, it is apparent that similar responses could be expected from increased humidity and the lower transpiration of the exposed plants. That dew on the leaves does reduce transpiration is to be expected on physical grounds and has been confirmed experimentally (Pisek and Cartellieri, 1939; Jones, 1957). Some in-

vestigators have, however, observed direct absorption of dew by plants (Gates, 1914; Michaelis and Michaelis, 1934; Rouschal, 1938, 1939; Arvidsson, 1951; Meidner, 1954; Waisel, 1958), and the previously reported experiments on water absorption confirm the occurrence of this phenomenon. The amount of water absorbed is frequently quite small, sometimes serving only to raise the leaf water content a few per cent (Wetzel, 1924; Krause, 1935), although on occasions it appears to be enough, in winter at any rate, to balance transpiration losses on the following day (Gates, 1914; Rouschal, 1938, 1939; Arvidsson, 1951).

If absorbed water is to make a contribution to the water economy of the plant, it would seem that it must be sufficient to cause greater growth or production than would otherwise be the case, or to enable plants to persist longer during protracted periods of dry weather. Direct evidence of the former factor is lacking, although Duvdevani's (1953, 1957) results of increased growth and production almost certainly reflect some direct effect of absorbed water. Evidence for the latter factor is much stronger (Stone and Fowells, 1955; Stone, Shachori and Stanley, 1956; Stone, 1957b). These authors have noticed that artificial dew at night can prolong the life of seedlings of *Pinus* spp. rooted in dry soil by a month and a half. As soil water levels did not change in these experiments, these results provide clear evidence of the direct effect of applied water in enabling resaturation of leaf tissues and maintenance of normal metabolic functions.

The effect of humid air on plant survival is less clear. Stone, Went and Young (1950) and Slatyer (1956a) have noticed absorption of unsaturated water vapour by some species when a favourable water potential gradient existed, and it is possible that such absorption could, to a limited extent, enable prolonged survival of plants under certain conditions. As Slatyer (1957a) has pointed out, however, there can be no real physiological benefit to the plant unless positive turgor pressure can be re-established in the active tissues. Thus the water potential of the water in the atmosphere has to be high enough to raise the plant water potential to a level above that at zero turgor pressure. If this occurs, it is conceivable that sufficient photosynthesis might proceed in early morning, before transpiration re-establishes severe water deficits, to enable the plant to balance its respiratory losses. In such instances, plants could possibly persist for some time without further re-charge of soil water.

In conclusion it may be stated that the primary significance of dew is probably through its effect in reducing transpiration and generally creating a more humid environment for plant growth. Although some absorption of dew appears to be of general occurrence, the amounts involved are usually small and it is difficult to separate the effect on the plant of this absorbed water and of the unabsorbed water on the leaf surfaces. In general, dew does appear to exert a favourable effect on plants, with the possible exception of its influence on the spread of some plant diseases, but it must be appreciated that, even in regions of heavy dewfall, the total amounts of dew received comprise only a very small proportion of the overall water requirements for active plant growth and development.

GENERAL SUMMARY

The main development in terminology in recent years has been a tendency to replace the established "pressure" terms and units with more basic terminology involving thermodynamic units. This has been motivated, in part, by the fact that the use of pressure terms and units can be misleading. For instance, high equivalent pressures or suctions can frequently be measured in soil or plant water systems, but these values represent potential energies and do not necessarily imply the existence of actual pressure differences. Furthermore, the simple and straightforward equation relating DPD to osmotic pressure and turgor pressure (DPD = OP–TP) refers ideally to the completely vacuolated plant cell. In tissues in which the non-vacuolar volume reaches significant proportions, additional factors may contribute to the free energy status of the water in the system.

An active controversy has been in progress as to the existence of a non-osmotic factor in active absorption. Following a group of early studies which provided strong evidence of a non-osmotic factor, it now appears that all this evidence can be reinterpreted in terms of standard osmotic theory. As a result, it is difficult at the present state of knowledge to identify a non-osmotic factor, even though it is apparent that active absorption is closely linked to, and immediately affected by, changes in metabolic activity, particularly in rate of respiration.

Of the two major processes involved in absorption, passive absorption is by far the more significant, almost all the water which passes through the plant being transported in this manner. In a discussion of water movement through plants, van den Honert (1948) and

others have shown the value of interpreting these phenomena through a consideration of the whole path of water movement through the soil-plant-atmosphere system. Van den Honert treated water movement through the system as a catenary process, the rate of which was controlled at the source of the greatest resistance. In addition, he applied an analog of Ohm's law to movement, so that the resistance was directly proportional to the reduction in free energy across any point in the system. This interpretation emphasizes the fact that the resistance in the gaseous phase, from sub-stomatal cavities to outside air, is far in excess of any other resistance that is likely to develop in a living plant. This provides the logical result that the stomates occur in the system at the only points where they could be of influence.

An important conclusion drawn from this analysis is that, because of the large resistance in the gaseous phase, resistance anywhere else in the plant can have little direct effect on transport. The mode of action of factors such as those which affect soil water availability or root permeability appears to be through reducing the water potential in the rest of the plant, with consequent reduced leaf water content, stomatal closure and retarded transpiration.

Of the factors affecting absorption, low soil water potential appears to be the most potent. It has a direct effect on the water potential in the plant, since the plant water potential must remain lower than the soil water potential in order to maintain an absorption gradient. It also has an indirect effect on root resistance through the effect of decreased hydration on permeability and suberization. Finally it has a direct effect on resistance to water movement through the soil-root surface zone, which influences the water potential gradient needed to maintain transport across this segment of the water transport path. It is probable that resistance to water movement through the soil can become of sufficient magnitude to parallel the resistance across the gaseous phase in the leaf and so directly influence transport. However, this could rarely occur in living plants, since a plant would die of dehydration before the resistance reached a high enough level.

Absorption of water by aerial organs and its subsequent transfer through the plant is also discussed in terms of the whole soil-plant-atmosphere system. Present knowledge indicates that the leaves of most plants can be expected to absorb limited quantities of water, either from liquid water or from saturated vapour, as long as a significant water potential gradient exists from atmosphere to leaf

favoring such movement. Most of the water appears to be absorbed through the cuticle, and the rate of absorption varies considerably with the wettability and surface characteristics of the epidermis. Subsequent transfer of water within the plant appears to depend primarily on the water potential gradients established, and if the gradient extends into the root medium, negative transport of water into the medium is sometimes observed. When the plant is rooted in soil, the general observation is that no net negative transport is found. Several explanations are offered for this phenomenon.

Application of studies dealing with absorption of water by leaves, and with negative transport, to the significance of dew and its possible importance to the water economy of the plant must be undertaken with caution. Although some absorption of dew appears probable under field conditions, the primary effects seem to be to minimise transpiration while the leaves are wet and generally to create a more humid environment for plant growth. This enables more rapid turgor recovery at night and permits photosynthesis in the early morning hours.

LITERATURE CITED

ADDOMS, R. M. 1946. Entrance of water into suberized roots of trees. Plant Physiol. **21**: 109-111.

ALDRICH, W. W., WORK, R. A., and LEWIS, M. R. 1935. Pear root concentration in relation to soil-moisture extraction in heavy clay soil. Jour. Agr. Res. **50**: 975-988.

ANDEL, O. M. VAN. 1952. Determinations of the osmotic value of exudation sap by means of the thermoelectric methods of Baldes and Johnson. Proc. Kon. Ned. Akad. Wet. **55**: 40-48.

————. 1953. The influence of salts on the exudation of tomato plants. Acta Bot. Néerl. **2**: 445-521.

ARCICHOVSKIJ, V., and OSSIPOV, A. 1931. Untersuchungen über die Saugkraft der Pflanzen. V. Die Saugkraft der baumartigen Pflanzen der zentralasiatischen Wüsten, nebst Transpirationmessungen am Saxaul (*Arthrophytum haloxylon* Litw.). Planta **14**: 552-565.

ARISZ, W. H., HELDER, R. J., and NIE, R. VAN. 1951. Analysis of the exudation process in tomato plants. Jour. Exp. Bot. **2**: 257-297.

ARMY, T. J., and KOZLOWSKI, T. T. 1951. Availability of soil moisture for active absorption in drying soil. Plant Physiol. **26**: 353-362.

ARVIDSSON, I. 1951. Austrocknungs- und Dürreresistenzverhältnisse einiger Repräsentanten öländischer Pflanzenvereine nebst Bemerkungen über Wasserabsorption durch oberirdische Organe. Oikos, Suppl. 1.

AYKIN, S. 1946. The relations between water permeability and suction potential in living and non-living osmotic systems. Rev. Fac. Sci. Univ. Istanbul **11**: 271-295.

BANGE, G. G. J. 1953. On the quantitative explanation of stomatal transpiration. Acta Bot. Néerl. **2**: 255-297.

BENNETT-CLARK, T. A. 1948. Non-osmotic water movement in plant cells. Disc. Faraday Soc. **3**: 134-139.

———— and BEXON, D. 1940. Water relations of plant cells. New Phytol. **39**: 337-361.

———— GREENWOOD, A. D., and BARKER, J. W. 1936. Water relations and osmotic pressure of plant cells. New Phytol. **35**: 277-291.

BERNSTEIN, L. GARDNER, W. R., and RICHARDS, L. A. 1959. Is there a vapor gap around plant roots. Science **129**: 1750-1753.

———— and HAYWARD, H. E. 1958. Physiology of salt tolerance. Ann. Rev. Plant Physiol. **9**: 25-46.

BERTRAND, A. R., and KOHNKE, H. 1957. Subsoil conditions and their effects on oxygen supply and the growth of corn roots. Proc. Soil. Sci. Soc. Amer. **21**: 135-140.

BIALOGLOWSKI, J. 1936. Effect of extent and temperature of roots on transpiration of rooted lemon cuttings. Proc. Amer. Soc. Hort. Sci. **34**: 96-102.

BLAIR, G. Y., RICHARDS, L. A., and CAMPBELL, R. B. 1950. The rate of elongation of sunflower plants and the freezing point of soil moisture in relation to permanent wilt. Soil Sci. **70**: 431-439.

BLOODWORTH, M. E., PAGE, J. B., and COWLEY, W. R. 1956. Some applications of the thermoelectric method for measuring water flow rates in plants. Agron. Jour. **48**: 222-228.

BOGEN, H. J. 1940. Ionenwirkung auf die Permeabilität von *Rhoeo discolor*. Zeit. Bot. **36**: 65-106.

————. 1941. Ionenwirkung auf die Permeabilität von *Gentiana cruciata*. Planta **32**: 150-175.

BONNER, J. 1959. Water transport. Science **129**: 447-450.

————, BANDURSKI, R. S., and MILLERD, A. 1953. Linkage of respiration to auxin-induced water intake. Physiol. Plant. **6**: 511-522.

BOON-LONG, T. S. 1941. Transpiration as influenced by osmotic concentration and cell permeability. Amer. Jour. Bot. **28**: 333-343.

BORMANN, F. H. 1957. Moisture transfer between plants through intertwined root systems. Plant Physiol. **32**: 48-55.

BOYNTON, D. 1954. Nutrition by foliar application. Ann. Rev. Plant Physiol. **5**: 31-54.

BRAUNER, L., and HASMAN, M. 1949. Über den Mechanismus der Heteroauxinwirkung auf die Wasseraufnahme von pflanzlichen Speichergewebe. Bull. Fac. Med. Istanbul **12**: 57-71.

———— and ————. 1952. Weitere Untersuchungen über den Wirkungsmechanismus des Heteroauxins bei der Wasseraufnahme von Pflanzenparenchymen. Protoplasma **41**: 302-326.

BREAZEALE, J. F., and CRIDER, F. J. 1934. Plant association and survival, and the build-up of moisture in semi-arid soils. Ariz. Agr. Exp. Sta., Tech. Bull 53.

———— and MCGEORGE, W. T. 1953a. Exudation pressure in roots of tomato plants under humid conditions. Soil Sci. **75**: 293-298.

———— and ————. 1953b. Influence of atmospheric humidity on root growth. Soil Sci. **76**: 361-365.

————, ————, and BREAZEALE, J. F. 1950. Moisture absorption by plants growing in an atmosphere of high humidity. Plant Physiol. **25**: 413-419.

————, ————, ————. 1951. Water absorption and transpiration by leaves. Soil Sci. **72**: 239-244.

BREWIG, A. 1936a. Die Regulationserscheinungen bei der Wasseraufnahme und die Wasserleitgeschwindigkeit in *Vicia faba*—Wurzeln. Jahrb. Wiss. Bot. **82**: 803-828.

————. 1936b. Beobachtungen über den Einfluss der Spross-Saugung auf die Stoffdurchlässigkeit der Wurzel. Ber. Deut. Bot. Ges. **54**: 80-85.

————. 1937. Permeabilitätsänderungen der Wurzelgewebe, die vom Spross aus beeinflusst werden. Zeit. Bot. **31**: 481-540.

————. 1939. Ausloesung leichter Wasserdurchlässigkeit an Wurzeln von *Vicia faba*. Planta **29**: 341-360.

BRIERLEY, W. G. 1934. Absorption of water by the foliage of some common fruit species. Proc. Amer. Soc. Hort. Sci. **32**: 277-283.

————. 1936. Further studies of the absorption of water by red raspberry foliage, and some evidence relative to the movement of water within the plant. Proc. Amer. Soc. Hort. Sci. **34**: 385-388.

BRIGGS, G. E. 1957. Some aspects of free space in plant tissues. New Phytol. **56**: 305-324.

BROUWER, R. 1953. Water absorption by the roots of *Vicia faba* at various transpiration strengths. I. Analysis of the uptake and the factors determining it. Proc. Kon. Ned. Akad. Wet. C. **56**: 106-115.

————. 1954. Water absorption by the roots of *Vicia faba* at various transpiration strengths. III. Changes in water conductivity artificially obtained. Proc. Kon. Ned. Akad. Wet. C. **57**: 68-80.

BROWN, E. M. 1939. Some effects of temperature on the growth and chemical composition of certain pasture grasses. Missouri Agr. Exp. Sta., Res. Bull. 299.

BROWN, R., and RICKLESS, P. 1949. A new method for the study of cell division and cell extension with some preliminary observations on the effect of temperature and nutrients. Proc. Roy. Soc. B. **136**: 110-125.

BROYER, T. C. 1947a. The movement of materials into plants. Part I. Osmosis and the movement of water into plants. Bot. Rev. **13**: 1-58.

————. 1947b. The movement of materials into plants. Part II. The nature of solute movement into plants. Bot. Rev. **13**: 125-167.

————. 1950. On the theoretical interpretation of turgor pressure. Plant Physiol. **25**: 135-139.

————. 1951a. An outline of energetics in relation to the movement of materials through a two-phased system. Plant Physiol. **26**: 598-610.

————. 1951b. Further theoretical considerations of modes of expression and factors possibly concerned in the movement of materials through a two-phased system. Plant Physiol. **26**: 655-676.

BRUECKNER, A. E. 1945. Transpiration studies on some Natal Midlands thornveld trees. So. Afr. Jour. Sci. **41**: 186-193.

BUCKINGHAM, E. 1907. Studies on the movement of soil moisture. U. S. Dept. Agr., Bur. Soils, Bull. 38.

BUFFEL, K. 1952. New techniques for comparative permeability studies on the oat coleoptile with reference to the mechanism of auxin action. Med. Kon. Vlaamse. Acad. Wet. België **14**: No. 7.

BURSTRÖM, H. 1941. Formative effects of carbohydrates on root growth. Bot. Not. **3**: 310-334.

————. 1953. Growth and water absorption of *Helianthus* tuber tissue. Physiol Plant. **6**: 685-691.

CAMERON, S. H. 1941. The influence of soil temperature on the rate of transpiration of young orange trees. Proc. Amer. Soc. Hort. Sci. **38**: 75-79.

CANNON, W. A. 1925. Physiological features of roots, with especial reference to the relation of roots to aeration of the soil. Carnegie Inst. Wash., Pub. 368.

CHANG, H. T., and LOOMIS, W. E. 1945. The effect of carbon dioxide on absorption of water and nutrients by roots. Plant Physiol. **20**: 221-232.

CHAPMAN, H. D., and PARKER, E. R. 1942. Weekly absorption of nitrate by young bearing orange trees growing out of doors in solution cultures. Plant Physiol. **17**: 366-376.

CHILDS, E. C., and COLLIS-GEORGE, N. 1948. Soil geometry and soil-water equilibria. Disc. Faraday Soc. **3**: 78-85.

———— and ————. 1950. The permeability of porous materials. Proc. Roy. Soc. A. **201**: 392-405.

CHUNG, C. H. 1935. A study of certain aspects of the phenomenon of transpiration periodicity. Ph. D. Diss., Ohio State Univ.

CLEMENTS, H. F., SHIGEURA, G., and AKAMINE, E. K. 1952. Factors affecting the growth of sugar cane. Univ. Hawaii Agr. Tech. Bull. 18.

COMMONER, B., FOGEL, S., and MULLER, W. H. 1943. The mechanism of auxin action. The effect of auxin on water absorption by potato tuber tissue. Amer. Jour. Bot. **30**: 23-28.

———— and MAZIA, D. 1942. The mechanism of auxin action. Plant Physiol. **17**: 682-685.

COOK, J. A., and BOYNTON, D. 1952. Some factors affecting the absorption of urea by McIntosh Apple leaves. Proc. Amer. Soc. Hort. Sci. **59**: 82-90.

COREY, A. T., and BLAKE, G. R. 1953. Moisture available to various crops in some New Jersey soils. Proc. Soil Sci. Soc. Amer. **17**: 314-330.

CRAFTS, A. S. 1933. Sulfuric acid as a penetrating agent in arsenical sprays for weed control. Hilgardia **8**: 125-147.

————, CURRIER, H. B. and STOCKING, C. R. (1949).—Water in the physiology of plants.

CRIDER, F. J. 1933. Selective absorption of ions not confined to young roots. Science **78**: 169.

CURRIER, H. B. 1944. Water relations of root cells of *Beta vulgaris*. Amer. Jour. Bot. **31**: 378-387.

DAVIS, C. H. 1940. Absorption of soil moisture by maize roots. Bot. Gaz. **101**: 791-805.

DAVSON, H., and DANIELLI, J. F. 1952. The permeability of natural membranes.

DAY, P. R. 1942. The moisture potential of soils. Soil Sci. **54**: 391-400.

DITTMER, H. J. 1937. A quantitative study of the roots and root hairs of a winter rye plant (*Secale cereale*). Amer. Jour. Bot. **24**: 417-420.

————. 1949. Root hair variations in plant species. Amer. Jour. Bot. **36**: 152-155.

DIXON, H. H. 1914. Transpiration and the ascent of sap in plants.

————. 1924. The transpiration stream.

DÖRING, B. 1935. Die Temperätureabhängigkeit der Wasseraufnahme und ihre ökologische Bedeutung. Zeits. Bot. **28**: 305-383.

DUVDEVANI, S. 1953. Dew gradients in relation to climate, soil and topography. Proc. UNESCO Desert Research Symp.: 136-152.

————. 1957. Dew research for arid agriculture. Discovery **18**: 330-334.

EATON, F. M. 1941. Water uptake and root growth as influenced by inequalities in the concentration of the substrate. Plant Physiol. **16**: 545-564.

————. 1942. Toxicity and accumulation of chloride and sulfate in plants. Jour. Agr. Res. **64**: 357-399.

————. 1943. The osmotic and vitalistic interpretations of exudation. Amer. Jour. Bot. **30**: 663-674.

EBELING, W. 1939. The rôle of surface tension and contact angle in the performance of spray liquids. Hilgardia **12**: 665-698.

EDLEFSEN, N. E. 1941. Some thermodynamic aspects of the use of soil-moisture by plants. Trans. Amer. Geophys. Union **22**: 917-940.

———— and ANDERSON, A.B.C. 1943. Thermodynamics of soil-moisture. Hilgardia **15**: 31-298.

———— and BODMAN, G. B. 1941. Field measurement of water movement through a silt loam soil. Jour. Amer. Soc. Agron. **33**: 713-731.

EISENZOPF, R. 1952. Ionenwirkungen auf die kutikuläre Wasseraufnahme von Koniferen. Phyton **4**: 149-159.

ELAZARI-VOLCANI, T. 1936. The influence of a partial interruption of the transpiration stream by root pruning and stem incisions on the turgor of citrus trees. Palestine Bot. Hort. Sci. **1**: 94-96.

ELLIS, C., and SWANEY, M. W. 1947. Soilless growth of plants.

EMERSON, W. W. 1954. Water conduction by severed grass roots. Jour. Agr. Sci. **45**: (2): 241-245.

EPSTEIN, E. 1956a. Mineral nutrition of plants: Mechanisms of uptake and transport. Ann. Rev. Plant Physiol. **7**: 1-24.

————. 1956b. Ion transport in plants. Science **124**: 1937.

ERICKSON, L. C. 1946. Growth of tomato roots as influenced by oxygen in the nutrient solution. Amer. Jour. Bot. **33**: 551-561.

ESAU, K. 1953. Plant anatomy.

EVANS, H. 1938. Studies on the absorbing surface of sugarcane root systems. I. Method of study with some preliminary results. Ann. Bot. (N.S.) **2**: 159-182.

FOGG, G. E. 1947. Quantitative studies on the wetting of leaves by water. Proc. Roy. Soc. B. **134**: 503-522.

FURR, J. R., and REEVE, J. O. 1945. The range of soil-moisture percentages through which plants undergo permanent wilting in some soils from semiarid irrigated areas. Jour. Agr. Res. **71**: 149-170.

GANONG, W. F. 1894. On the absorption of water by the green parts of plants. Bot. Gaz. **19**: 136-143.

GATES, F. 1914. Winter as a factor in the xerophily of certain evergreen ericads. Bot. Gaz. **57**: 445-489.

GAUCH, H. G. 1957. Mineral nutrition of plants. Ann. Rev. Plant Physiol. **8**: 31-64.

GIER, L. J. 1940. Root systems of bright belt tobacco. Amer. Jour. Bot. **27**: 780-787.

GESSNER, F. 1956. Die Wasseraufnahme durch Blätter und Samen. Encyc. Plant Physiol. Vol. **3**: 215-246.

GILL, W. R., and MILLER, R. D. 1956. A method for study of the influence of mechanical impedance and aeration on the growth of seedling roots. Soil Sci. Soc., Proc. **20**: 154-157.

GILLILAND, E. R. 1938. Fundamentals of drying and airconditioning. Ind. Eng. & Chem. **30**: 506-514.

GINGRICH, J. R., and RUSSELL, M. B. 1956. Effect of soil moisture tension and oxygen concentration on the growth of corn roots. Agron. Jour. **48**: 517-520.

———— and ————. 1957. A comparison of effects of soil moisture tension and osmotic stress on root growth. Soil Sci. **84**: 185-194.

GINSBURG, J. M. 1930. Studies on penetration of oils into plant tissue. N. J. Agr. Exp. Sta., Ann. Rep. **51**: 163-167.

GRADMANN, H. 1928. Untersuchungen über die Wasserverhältnisse des Bodens als Grundlage des Pflanzenwachstums. Jahrb. Wiss. Bot. **69**: 1-100.

GREENIDGE, K. N. H. 1955a. Observations on the movement of moisture in large woody stems. Canad. Jour. Bot. **33**: 202-221.

————. 1955b. Studies in the physiology of forest trees. III. The effect of drastic interruption of conducting tissues on moisture movement. Amer. Jour. Bot. **42**: 582-587.

————. 1957. Ascent of sap. Ann. Rev. Plant Physiol. **8**: 237-256.

————. 1958. A note on the rates of upward travel of moisture in trees under differing experimental conditions. Canad. Jour. Bot. **36**: 357-361.

GREGORY, F. G., MILTHORPE, F. L., PEARSE, H. L., and SPENCER, H. J. 1950a. Experimental studies of the factors controlling transpiration. I. Apparatus and experimental technique. Jour. Exp. Bot. **1**: 1-14.

————, ————, ———— and ————. 1959b. Experimental studies of the factors controlling transpiration. II. The relation between transpiration rate and leaf water content. Jour. Exp. Bot. **1**: 15-28.

GROSSENBACHER, K. A. 1939. Autonomic cycle of rate of exudation of plants. Amer. Jour. Bot. **26**: 107-109.

GUEST, P. L., and CHAPMAN, H. D. 1949. Investigations on the use of iron sprays, dusts, and soil applications to control iron chlorosis of citrus. Proc. Amer. Soc. Hort. Sci. **54**: 11-21.

GURR, C. G., MARSHALL, T. J., and HUTTON, J. T. 1952. Movement of water in soil due to a temperature gradient. Soil Sci. **74**: 335-345.

HAAS, A. R. C. 1936. Growth and water losses in citrus as affected by soil temperature. Calif. Citrograph **21**: 467, 469.

HACKETT, D. P. 1952. The osmotic change during auxin-induced water uptake by potato tissue. Plant Physiol. **27**: 279-284.

———— and THIMANN, K. V. 1952. The nature of the auxin-induced water uptake by potato tissue. Amer. Jour. Bot. **39**: 553-560.

———— and ————. 1953. The nature of the auxin-induced water uptake by potato tissue. II. The relation between respiration and water absorption. Amer. Jour. Bot. **40**: 183-188.

HAGAN, R. M. 1949. Autonomic diurnal cycles in the water relations of non-exuding detopped root systems. Plant Physiol. **24**: 441-454.

————. 1950. Soil aeration as a factor in water absorption by the roots of transpiring plants. Plant Physiol. **25**: 748-762.

————. 1952a. Temperature and growth processes. *In*: "Soil physical conditions and plant growth".

————. 1952b. Soil temperature and plant growth. *In*: "Soil physical conditions and plant growth".

HAINES, F. M. 1952. The absorption of water by leaves in an atmosphere of high humidity. Jour. Exp. Bot. **3**: 95-98.

————. 1953. The absorption of water by leaves in fogged air. Jour. Exp. Bot. **4**: 106-107.

HANDLEY, W. R. C. 1939. The effect of prolonged chilling on water movement and radial growth in trees. Ann. Bot. **3**: 803-813.

HARRIS, D. G., and VAN BAVEL, C. H. M. 1957. Growth, yield and water absorption of tobacco plants as affected by the composition of the root atmosphere. Agron. Jour. **49**: 11-14.

HAYWARD, H. E., BLAIR, W. M., and SKALING, P. E. 1942. Device for measuring entry of water into roots. Bot. Gaz. **104**: 152-160.

———— and SPURR, W. B. 1943. Effects of osmotic concentration of substrate on the entry of water into corn roots. Bot. Gaz. **105**: 152-164.

———— and ————. 1944. Effects of isosmotic concentrations of inorganic and organic substrates on entry of water into corn roots. Bot. Gaz. **106**: 131-139.

HENDRICKSON, A. H., and VEIHMEYER, F. J. 1929. Irrigation experiments with peaches in California. Calif. Agr. Exp. Sta., Bull. **479**: 1-56.

———— and ————. 1931. Influence of dry soil on root extension. Plant Physiol. **6**: 567-576.

———— and ————. 1934. Irrigation experiments with prunes. Calif. Agr. Exp. Sta., Bull. 573.

———— and ————. 1941. Moisture distribution in soil containers. Plant Physiol. **4**: 821-826.

———— and ————. 1942. Irrigation experiments with pears and apples. Calif. Agr. Exp. Sta., Bull. 667.

HILGEMAN, R. H. 1948. Changes in soil moisture in the top eight feet of bare soil during 22 months after wetting. Jour. Amer. Soc. Agron. **40**: 919-925.

HOAGLAND, D. R., and BROYER, T. C. 1942. Accumulation of salt and permeability in plant cells. Jour. Gen. Physiol. **25**: 865-880.

———— and ————. 1942. General nature of the process of salt accumulation by roots with description of experimental methods. Plant Physiol. **11**: 471-507.

HOHN, K. 1954. Untersuchungen über das Wasserdampfaufnahme- und Wasserdampfabgabe-Vermögen höherer Landpflanzen. Beitr. Biol. Pflanz. **30**: 159-178.

HONERT, T. H. VAN DEN 1948. Water transport in plants as a catenary process. Disc. Faraday Soc. **3**: 146-153.

HUNT, F. M. 1951. Effects of flooded soil on growth of pine seedlings. Plant Physiol. **26**: 363-368.

HUNTER, A. S., and KELLEY, O. J. 1946. The extension of plant roots into dry soil. Plant Physiol. **21**: 445-451.

HYGEN, G. 1953. Studies in plant transpiration. II. Physiol. Plant. **6**: 106-131.

JANES, B. E. 1954. Absorption and loss of water by tomato leaves in a saturated atmosphere. Soil Sci. **78**: 189-197.

JANNTI, A. 1953. Grassland practices in relation to soil water in central, west, and north European countries. Acta Agr. Fenn. **81**(6): 1-39.

———— and KRAMER, P. J. 1956. Regrowth of pastures in relation to soil moisture and defoliation. Proc. 7th Int. Grass Conf. Vol. **1**(2): 1-11.

JOHNSTON, R. D. 1959. Control of water movement by stem chilling. Austral. Jour. Bot. **7**: 97-108.

JONES, R. L. 1957. The effect of surface wetting on the transpiration of leaves. Physiol. Plant. **10**: 281-288.

KAUFMAN, C. M. 1945. Root growth of jack pine on several sites in the Cloquet Forest, Minnesota. Ecology **26**: 10-23.

KAUSCH, W. 1955. Saugkraft und Wassernachleitung im Boden als physiologische Faktoren. Unter besonderer Berücksichtigung des Tensiometers. Planta **45**: 217-263.

KELLEY, V. W. 1930. Effect of certain hydrocarbon oils on respiration of foliage and dormant twigs of the apple. Illinois Agr. Exp. Sta., Bull. **348**: 371-406.

KELLY, S. 1947. The relations between respiration and water uptake in the oat coleoptile. Amer. Jour. Bot. **34**: 521-526.

KETELLAPPER, H. J. 1953. The mechanism of the action of indole-3-acetic acid on the water absorption by *Avena* coleoptile sections. Acta Bot. Néerl. **2**: 387-444.

KLEMM, G. 1956. Untersuchungen über den Transpirationswiderstand der Mesophyllmembranen und seine Bedeutung als Regulator für die stomatäre Transpiration. Planta **47**: 547-587.

KLUTE, A. 1952. A numerical method for solving the flow equation for water in unsaturated materials. Soil Sci. **73**: 105-116.

KMOCK, H. G., RAMIG, R. E., FOX, R. L., and KOEHLER, F. E. 1957. Root development of winter wheat as influenced by soil moisture and nitrogen fertilization. Agron. Jour. **49**: 20-25.

KNIGHT, H. J., CHAMBERLAIN, J. C., and SAMUELS, C. D. 1929. On some limiting factors in the use of saturated petroleum oils as insecticides. Plant Physiol. **4**: 299-321.

KORVEN, H. C., and TAYLOR, S. A. 1959. The Peltier effect and its use for determining relative activity of soil water. Canad. Jour. Soil. Sci. **39**: 76-85.

KOZLOWSKI, T. T. 1943. Transpiration rates of some forest tree species during the dormant season. Plant Physiol. **18**: 252-260.

———— and SCHOLTES, W. 1948. Growth of roots and root hairs of pine and hardwood seedlings in the Piedmont. Jour. Forestry **46**: 750-754.

KRAMER, P. J. 1932. The absorption of water by root systems of plants. Amer. Jour. Bot. **19**: 148-164.

————. 1933. The intake of water through dead root systems and its relation to the problem of absorption by transpiring plants. Amer. Jour. Bot. **20**: 481-492.

————. 1937. The relation between rate of transpiration and rate of absorption of water in plants. Amer. Jour. Bot. **24**: 10-15.

————. 1938. Root resistance as a cause of the absorption lag. Amer. Jour. Bot. **25**: 110-113.

————. 1940a. Root resistance as a cause of decreased water absorption by plants at low temperatures. Plant Physiol. **15**: 63-79.

————. 1940b. Causes of decreased absorption of water by plants in poorly aerated media. Amer. Jour. Bot. **27**: 216-220.

————. 1941. Soil moisture as a limiting factor for active absorption and root pressure. Amer. Jour. Bot. **28**: 446-451.

————. 1942. Species differences with respect to water absorption at low soil temperatures. Amer. Jour. Bot. **29**: 828-832.

————. 1945. Absorption of water by plants. Bot. Rev. **11**: 310-355.

————. 1946. Absorption of water through suberized roots of trees. Plant Physiol. **21**: 37-41.

————. 1949. Plant and soil water relationships.

————. 1950. Effects of wilting on the subsequent intake of water by plants. Amer. Jour. Bot. **37**: 280-284.
————. 1951. Causes of injury to plants resulting from flooding of the soil. Plant Physiol. **26**: 722-736.
————. 1955a. Water relations of plant cells and tissues. Ann. Rev. Plant Physiol. **6**: 253-272.
————. 1955b. Water content and water turnover in plant cells. Encyc. Plant Physiol. Vol. 1: 194-222.
————. 1956a. The uptake of water by plant cells. Encyc. Plant. Physiol. Vol. 2: 316-336.
————. 1956b. Permeability in relation to respiration. Encyc. Plant Physiol. Vol. 2: 358-368.
————. 1956c. Physical and physiological aspects of water absorption. Encyc. Plant Physiol. Vol. 3: 124-159.
————. 1956d. Roots as absorbing organs. Encyc. Plant Physiol. Vol. 3: 188-214.
————. 1957. Outer space in plants. Science **125**: 633-635.
———— and Coile, T. S. 1940. An estimate of the volume of water made available by root extension. Plant Physiol. **15**: 743-747.
———— and Jackson, W. T. 1954. Causes of injury to flooded tobacco plants. Plant Physiol. **29**: 241-245.
Krause, H. 1935. Beiträge zur Kenntnis der Wasseraufnahme durch oberirdische Pflanzenorgane. Österr. Bot. Zeits. **84**: 241-270.
Kuiper, P. J. C., and Bierhuizen, J. F. 1958. The effect of some environmental factors on the transpiration of plants under controlled conditions. Med. Landb. Hoogesch. Wageningen **58**: 1-16.
Lachenmeier, J. 1932. Transpiration und Wasserabsorption intaker Pflanzen nach vorausgegangener Verdunkelung bei Konstanz der Lichtintensität und der übrigen Aussenfaktoren. Jahrb. Wiss. Bot. **76**: 765-827.
Leonard, O. A. 1944. Use of root pressures in determining injury to roots by cultivation. Plant Physiol. **19**: 157-163.
———— and Pinckard, J. A. 1946. Effect of various oxygen and carbon dioxide concentrations on cotton root development. Plant Physiol. **21**: 18-36.
Levitt, J. 1941. Frost killing and hardiness of plants.
————. 1947. The thermodynamics of active (non-osmotic) water absorption. Plant Physiol. **22**: 514-525.
————. 1953. Further remarks on the thermodynamics of active (non-osmotic) water absorption. Physiol. Plant. **6**: 240-252.
————. 1954a. Do plants absorb water against a gradient. VIII Int. Bot. Congr. **11**: 213-215.
————. 1954b. Steady state versus equilibrium thermodynamics in the concept of "Active" water absorption. Physiol. Plant. **7**: 592-594.
———— and Scarth, G. W. 1936. Frost-hardening studies with living cells. II. Permeability in relation to frost resistance and the seasonal cycle. Canad. Jour. Res. C. **14**: 285-305.
————, ———— and Gibbs, R. D. 1936. Water permeability of isolated protoplasts in relation to volume change. Protoplasma **26**: 237-248.
Lewis, F. J. 1945. Physical condition of the surface of the mesophyll cell walls of the leaf. Nature **156**: 407-490.
————. 1948. Water movement in leaves. Disc. Faraday Soc. **3**: 159-162.

LEWIS, G. N. and RANDALL, M. 1923. Thermodynamics and the free energy of chemical substances.

LIVINGSTON, B. E. 1913. The resistance offered by leaves to transpirational water loss. Plant World **16**: 1-35.

LONG, E. M. 1943. The effect of salt additions to the substrate on intake of water and nutrients by roots of approach-grafted tomato plants. Amer. Jour. Bot. **30**: 594-601.

MACDOUGAL, D. T. 1926. The hydrostatic system of trees.

MAGISTAD, O. C. 1945. Plant growth on saline and alkali soils. Bot. Rev. **11**: 181-230.

MARLOTH, R. 1909. Notes on the absorption of water by aerial organs of plants. Trans. Roy. Soc. So. Africa **1**: 429-433.

————. 1926. Weitere Beobachtungen über die Wasser der Pflanzen durch oberirdische Organe. Ber. Deut. Bot. Ges. **44**: 448-455.

MARTIN, E. V. 1940. Effect of soil moisture on growth and transpiration in *Helianthus annuus*. Plant Physiol. **15**: 449-466.

MASON, T. G., and PHILLIS, E. 1939. Experiments on the extraction of sap from the vacuole of the leaf of the cotton plant and their bearing on the osmotic theory of water absorption of the cell. Ann. Bot. (N.S.) **3**: 531-544.

MCDERMOTT, J. J. 1945. The effect of the moisture content of the soil upon the rate of exudation. Amer. Jour. Bot. **32**: 570-574.

MAXIMOV, N. A. 1929. The plant in relation to water.

MEES, G. C., and WEATHERLEY, P. E. 1957a. The mechanism of water absorption by roots. I. Preliminary studies on the effects of hydrostatic pressure gradients. Proc. Roy. Soc. B. **147**: 367-380.

———— and ————. 1957b. The mechanism of water absorption by roots. II. The role of hydrostatic pressure gradients across the cortex. Proc. Roy. Soc. B. **147**: 381-391.

MEIDNER, H. 1954a. Measurements of water intake from the atmosphere by leaves. New Phytol. **53**: 423-426.

————. 1955. Changes in the resistance of the mesophyll tissue with changes in the leaf water content. Jour. Exp. Bot. **6**: 94-99.

MENDEL, K. 1944. Orange leaf transpiration under orchard conditions. II. Soil moisture content decreasing. Palestine Jour. Bot. (R) **5**: 59-85.

MERCER, F. V. 1955. The water relations of plant cells. Proc. Linn. Soc. New So. Wales **80**: 6-29.

MEYER, B. S. 1938. The water relations of plant cells. Bot. Rev. **4**: 531-547.

————. 1945. A critical evaluation of the terminology of diffusion phenomena. Plant Physiol. **20**: 142-164.

————. 1956. Wall and turgor pressure and tension. Diffusion pressure defiit or suction force. Encyc. Plant Physiol. Vol. **2**: 38-56.

MICHAELIS, P. 1934. Ökologische Studien an der alpinen Baumgrenze. IV. Zur Kenntnis des winterlichen Wasserhaushaltes. Jahrb. Wiss. Bot. **80**: 169-247.

MILTHORPE, F. L. 1959. Transpiration from crop plants. Field Crop Abstracts **12**(1): 1-9.

———— and SPENCER, E. 1957. Experimental studies of the factors controlling transpiration. Jour. Exp. Bot. **8**: 413-437.

MONSELISE, S. P., and HAGIN, J. 1955. Influence of soil aggregation on the rooting of carnation cuttings. Plant & Soil **6**: 245-250.

MONTEITH, J. L., and OWEN, P. C. 1958. A thermocouple method for measur-

ing relative humidity in the range 95-100%. Jour. Sci. Instr. **35**: 443-446.

MYERS, G. M. P. 1951. The water permeability of unplasmolysed tissues. Jour. Exp. Bot. **2**: 129-144.

NAKAJIMA, Y. 1928. Wirkung des der Luft entleerten Wassers auf die Wasseraufnahme verschiedener Körper. Sci. Rep. Tôhoku. Imp. Univ. IV. Biol. **3**: 279-298.

NIGHTINGALE, G. T. 1935. Effects of temperature on growth, anatomy and metabolism of apple and peach roots. Bot. Gaz. **96**: 581-639.

NUTMAN, F. J. 1934. The root-system of Coffea arabica. III. The spatial distribution of the absorbing area of the root. Emp. Jour. Exp. Agr. **2**: 293-302.

OPPENHEIMER, H. R. 1951. Physiological behavior of maize under irrigation. Palestine Jour. Bot. R **8**: 32-44.

ORDIN, L., APPLEWHITE, T. H., and BONNER, J. 1956. Auxin-induced water uptake by *Avena* coleoptile sections. Plant Physiol. **31**: 44-53.

———— and BONNER, J. 1956. Permeability of *Avena* coleoptile sections to water measured by diffusion of deuterium hydroxide. Plant Physiol. **31**: 53-57.

———— and JACOBSON, L. 1955. Inhibition of ion absorption and respiration in barley roots. Plant Physiol. **30**: 21-27.

———— and KRAMER, P. J. 1956. Permeability of Vicia Faba root segments to water as measured by diffusion of deuterium hydroxide. Plant Physiol. **31**: 468-471.

OVERBEEK, J. VAN. 1942. Water uptake by excised root systems of the tomato due to non-osmotic forces. Amer. Jour. Bot. **29**: 677-682.

————. 1944. Auxin water uptake and osmotic pressure in potato tissue. Amer. Jour. Bot. **31**: 265-269.

OWEN, P. C. 1952. The relation of water absorption by wheat seeds to water potential. Jour. Exp. Bot. **3**: 276-296.

PALMITER, D. H., ROBERTS, E. A., and SOUTHWICK, M. D. 1946. Apple leaf structure in relation to penetration by spray solutions. Phytopath. **36**: 681.

PARKER, J. 1949. Effects of variations in the root leaf ratio on transpiration rate. Plant Physiol. **24**: 739-743.

————. 1950. The effects of flooding on the transpiration and survival of some south-eastern forest tree species. Plant Physiol. **25**: 453-460.

PETERS, D. B. 1957. Water uptake of corn roots as influenced by soil moisture content and soil moisture tension. Soil Sci. Soc. Amer., Proc. **21**: 481-484.

PHILIP, J. R. 1954. Some recent advances in hydrologic physics. Jour. Inst. Eng. Australia **26**: 255-259.

————. 1955. The concept of diffusion applied to soil water. Proc. Nat. Acad. [India] A. **211**: 93-104.

————. 1957a. The physical principles of soil water movement during the irrigation cycle. Proc. III. Int. Congr. Irrig. Drainage **8**: 125-154.

————. 1957b. The theory of infiltration. I. The infiltration equation and its solution. Soil Sci. **83**: 345-357.

————. 1958a. Physics of water movement in porous solids. Highway Res. Board, Washington, Spec. Rep. 40.

————. 1958b. The osmotic cell, solute diffusibility, and the plant water economy. Plant Physiol. **33**: 264-271.

———. 1958c. Osmosis and diffusion in tissue: Half-times and internal gradients. Plant Physiol. **33**: 275-278.

——— and VRIES, D. A. DE. 1957. Moisture movement in porous materials under temperature gradients. Trans. Amer. Geophys. Union **38**: 222-232.

PISEK, A. and CARTELLIERI, E. 1939. Zur Kenntnis des Wasserhaushaltes der Pflanzen. IV. Bäume und Sträucher. Jahrb. Wiss. Bot. **88**: 22-68.

POSTLETHWAIT, S. N., and ROGERS, B. 1958. Tracing the path of the transpiration stream in trees by the use of radio-active isotopes. Amer. Jour. Bot. **45**: 753-757.

PRESTON, R. D. 1952. Movement of water in higher plants. *In*: "Deformation and flow in biological systems".

———. 1954. The transpiration of plants. Proc. Leeds Phil. & Lit. Soc. **6**: 154-167.

REED, J. F. 1939. Root and shoot growth of shortleaf and loblolly pines in relation to certain environmental conditions. Duke Univ., School Forestry, Bull. 4.

REINDERS, D. E. 1938. The process of water-intake by discs of potato tuber tissue. Proc. Koni. Akad. Wet. Amsterdam **41**: 820-831.

———. 1942. Intake of water by parenchymatic tissue. Rec. Trav. Bot. Néerl. **39**: 1-140.

RENNER, O. 1911. Experimentelle Beiträge zur Kenntnis der Wasserbewegung. Flora **103**: 171-247.

———. 1912. Versuche zur Mechanik der Wasserversorgung. I. Der Druck in den Leitungsbahnen von Freilandpflanzen. Ber. Deut. Bot. Ges. **30**: 576-580.

———. 1915. Die Wasserversorgung der Pflanzen. Handwörterbuch Naturwiss. **10**: 538-557.

———. 1929. Versuche zur Bestimmung des Filtrationswiderstandes der Wurzeln. Jahrb. Wiss. Bot. **70**: 805-838.

RICHARDS, L. A. 1936. Capillary conductivity data for three soils. Jour. Amer. Soc. Agron. **28**: 297-300.

——— and LOOMIS, W. E. 1942. Limitations of auto-irrigators for controlling soil moisture under growing plants. Plant Physiol. **17**: 223-235.

——— and MOORE, D. C. 1952. Influence of capillary conductivity and depth of wetting on moisture retention in soil. Trans. Amer. Geophys. Union. **22**: 531-540.

——— and OGATA, GEN 1958. Thermocouple for vapour pressure measurements in biological and soil systems at high humidity. Science **128**: 1089-1090.

——— and WADLEIGH, C. H. 1952. Soil water and plant growth. *In*: "Soil physical conditions and plant growth".

——— and WEAVER, L. R. 1944. Moisture retention by some irrigated soils as related to soil-moisture tension. Jour. Agr. Res. **69**: 215-235.

——— and WILSON, B. D. 1936. Capillary conductivity measurement in peat soils. Jour. Amer. Soc. Agron. **28**: 427-431.

ROBERTS, E. A., Southwick, M. D. and PALMITER, D. H. 1948. A microchemical examination of McIntosh apple leaves showing relationship of cell wall constituents to penetration of spray solutions. Plant Physiol. **23**: 557-559.

ROBERTS, O., and STYLES, S. A. 1939. An apparent connection between the

presence of colloids and the osmotic pressures of conifer leaves. Sci. Proc. Roy. Dublin Soc. **22**: 119-125.

ROBERTSON, R. N. 1958. The uptake of minerals. Encyc. Plant Physiol. Vol. 4: 243-279.

ROHRBAUGH, P. W. 1934. Penetration and accumulation of petroleum spray oils in the leaves, twigs and fruit of citrus trees. Plant Physiol. **9**: 699-730.

ROLLINS, R. L., SPANGLER, M. G., and KIRKHAM, D. 1954. Movement of soil moisture under a thermal gradient. Highway Res. Board Proc. **33**: 492-508.

RØNNIKE, F. 1957. On the growth of roots and hypocotyls of lupine plants cultivated in substrata with different water contents. Physiol. Plant. **10**: 440-444.

ROSENE, H. F. 1937. Distribution of the velocities of absorption of water in the onion root. Plant Physiol. **12**: 1-19.

————. 1941. Comparison of rate of water intake in contiguous regions of intact and isolated roots. Plant Physiol. **16**: 19-38.

————. 1943. Quantitative measurement of the velocity of water absorption in individual root hairs by a microtechnique. Plant Physiol. **18**: 588-607.

————. 1944. Effect of cyanide on rate of exudation in excised onion roots. Amer. Jour. Bot. **31**: 172-174.

————. 1947. A bibliography of continuous bioelectric currents and bioelectric fields in animals and plants. *In*: "Bioelectric fields and growth".

————. 1950. Effect of anoxia on water exchange and oxygen consumption of onion root tissues. Jour. Cell. & Comp. Physiol. **35**: 179-193.

————. 1954. The water absorptive capacity of root hairs. VIII. Int. Bot. Congr. **11**: 217-218.

———— and BARTLETT, L. E. 1950. Effect of anoxia on water influx of individual radish root hair cells. Jour. Cell. & Comp. Physiol. **36**: 83-96.

———— and WALTHALL, A. M. J. 1954. Comparison of the velocities of water influx into young and old root hairs of wheat seedlings. *Physiol. Plant.* **7**: 190-194.

ROUSCHAL, E. 1938. Zur Ökologie der Macchien. I. Jahrb. Wiss. Bot. **87**: 436-523.

————. 1939. Beiträge zum winterlichen Wasserhaushalt von *Cheiranthus cheiri* und anderer wintergrünen Gartenpflanzen. Öster. Bot. Zeits. **88**: 148-154.

RUHLAND, W. 1955. Encyclopedia of Plant Physiology. Vol. 1.

————. 1956a. Encyclopedia of Plant Physiology. Vol. 2.

————. 1956b. Encyclopedia of Plant Physiology. Vol. 3.

RUSSELL, M. B. 1952. Soil aeration and plant growth. *In:* "Soil physical conditions and plant growth".

———— and DANIELSON, R. E. 1956. Time and depth patterns of water use by corn. Agron. Jour. **48**: 163-165.

————, DAVIS, F. E. and BLAIR, R. A. 1940. The use of tensiometers for following soil moisture conditions under corn. Jour. Amer. Soc. Agron. **32**: 922-930.

SANDSTROM, B. 1950. The ion absorption in roots lacking epidermis. Physiol. Plant. **3**: 496-505.

SCHOLANDER, P. F., LOVE, W. E., and KANWISHER, J. W. 1955. The rise of sap in tall grapevines. Plant Physiol. **30**(2): 93-104.

———, Ruud, B., and Leivestad, H. 1957. The rise of sap in a tropical liana. Plant Physiol. **32**: 1-6.

Schroeder, R. A. 1939. The effect of root temperature upon the absorption of water by the cucumber. Missouri Agr. Exp. Sta., Res. Bull. 309.

Scott Russell, R., and Shorrocks, V. M. 1959. The relationship between transpiration and the absorption of inorganic ions by intact plants. Jour. Exp. Bot. **10**: 301-316.

Shaw, B. T. 1952. Soil physical conditions and plant growth.

Sierp, H., and Brewig, A. 1935. Quantitative Untersuchungen über die Wasserabsorptionszone der Wurzeln. Jahrb. Wiss. Bot. **82**: 99-122.

Slatyer, R. O. 1956a. Absorption of water from atmospheres of different humidity and its transport through plants. Aust. Biol. Sci. **9**: 552-558.

———. 1956b. Evapotranspiration in relation to soil moisture. Neth. Jour. Agr. Sci. **4**: 73-76.

———. 1957a. The influence of progressive increases in total soil moisture stress on transpiration, growth and internal water relationships of plants. Aust. Jour. Biol. Sci. **10**: 320-336.

———. 1957b. The significance of the permanent wilting percentage in studies of plant and soil water relations. Bot. Rev. **23**: 585-636.

——— and Taylor, S. A. 1960. Terminology in plant-soil water relations. Nature [in press].

Spanner, D. C. 1951. The Peltier effect and its use in the measurement of suction pressure. Jour. Exp. Bot. **11**: 145-168.

———. 1952. The suction potential of plant cells and some related topics. Ann. Bot. (N.S.) **16**: 379-407.

———. 1954. The thermodynamics of actively-maintained turgor pressure, with a note on the idea of permeability. Physiol. Plant. **7**: 278-282.

Staple, W. V., and Lehane, J. J. 1954. Movement of water in unsaturated soils. Canad. Jour. Agr. Sci. **34**: 329-342.

Steubing, L. 1949. Beiträge zur Tauwasseraufnahme höherer Pflanzen. Biol. Zentr. **68**: 252-259.

Steward, F. C., Stout, P. R., and Preston, C. 1940. The balance sheet of metabolites for potato discs showing the effect of salts and dissolved oxygen on metabolism at 23°C. Plant Physiol. **15**: 409-447.

Stiles, W. 1956. Water relations of the plant cell. Sci. Progr. **44**: 686-696.

Stocker, O.u. Kausch, W. 1952. Bodenfeuchte und Tensiometermessung. Ber. Deut. Wetterdienst U. S. Zone **32**: 15-18.

Stocking, C. R. 1945. The calculations of tensions in Cucurbita pepo. Amer. Jour. Bot. **32**: 126-134.

———. 1956. Osmotic pressure or osmotic value. Encyc. Plant Physiol. Vol. 2: 57-70.

Stone, E. C. 1957a. Dew as an ecological factor. I. A review of the literature. Ecology **38**: 407-413.

———. 1957b. Dew as an ecological factor. II. The effect of artificial dew on the survival of *Pinus ponderosa* and associated species. Ecology **38**: 414-422.

——— and Fowells, H. A. 1955. Survival value of dew as determined under laboratory conditions with *Pinus ponderosa*. Forest Science **1**: 183-188.

———, Shachori, A. Y., and Stanley, R. G. 1956. Water absorption

by needles of *ponderosa* pine seedlings and its internal redistribution. Plant Physiol. **31**: 120-126.

————, WENT, F. W., and YOUNG, C. L. 1950. Water absorption from the atmosphere by plants growing in dry soil. Science **111**: 546-548.

STUCKEY, I. H. 1941. Seasonal growth of grass roots. Amer. Jour. Bot. **28**: 486-491.

STRUGGER, S. 1943. Der aufsteigende Saftstrom in der Pflanze. Naturwiss. **31**: 181-194.

————. 1949. Praktikum der Zell- und Gewebephysiologie der Pflanze.

TAYLOR, S. A., and CAVAZZA, L. 1954. The movement of soil moisture in response to temperature gradients. Soil Sci Soc. Amer. Proc. **18**: 351-358.

TURRELL, F. M. 1947. Citrus leaf stomata : Structure, composition and pore size in relation to penetration of liquids. Bot. Gaz. **108**: 476-483.

URSPRUNG, A., and BLUM, G. 1916. Über die Verteilung des osmotischen Wertes in der Pflanze. Ber. Deut. Bot. Ges. **34**: 88-104.

VEIHMEYER, F. J., and HENDRICKSON, A. H. 1927. Soil moisture conditions in relation to plant growth. Plant Physiol. **2**: 71-82.

———— and ————. 1928. Soil moisture at permanent wilting of plants. Plant Physiol. **3**: 350-357.

———— and ————. 1934. Some plant and soil moisture relationships. Bull. Amer. Soil Survey Assoc. **15**: 76-80.

———— and ————. 1938. Soil moisture as an indication of root distribution in deciduous orchards. Plant Physiol. **13**: 169-177.

———— and ————. 1946. Soil density as a factor in determining the permanent wilting percentage. Soil Sci. **62**: 451-456.

———— and ————. 1948. Soil density and root penetration. Soil Sci. **65**: 487-493.

———— and ————. 1949. Methods of measuring field capacity and permanent wilting percentage of soils. Soil Sci. **68**: 75-94.

VOLK, G. M. 1947. Significance of moisture translocation from soil zones of low moisture tension to zones of high moisture tension by plant roots. Jour. Amer. Soc. Agron. **39**: 93-106.

WAISEL, Y. 1958. Dew absorption by plants of arid zones. Bull. Res. Council Israel **6D**: 180-186.

WALTER, H. 1936. Die ökologischen Verhältnisse in der Namib-Nebelwüste. (Südwestafrika). Jahrb. Wiss. Bot. **84**: 58-222.

————. 1951. Einführung in die Phytologie. III. Grundlagen der Pflanzenverbreitung. 1.

————. 1955. The water economy and hydrature of plants. Ann. Rev. Plant Physiol. **6**: 239-252.

WEATHERLEY, P. E. 1951. Studies of the water relations of the cotton plant. II. Diurnal and seasonal fluctuations and environmental factors. New Phytol. **50**: 36-51.

———— and SLATYER, R. O. 1957. Relationship between relative turgidity and diffusion pressure deficit in leaves. Nature **179**: 1085-1086.

WEAVER, J. E., and ZINK, E. 1946. Annual increase of underground materials in three range grasses. Ecology **277**: 115-127.

WENT, F. W. 1944. Plant growth under controlled conditions. III. Correlation between various physiological processes and growth in the tomato plant. Amer. Jour. Bot. **31**: 597-618.

WETZEL, K. 1924. Die Wasseraufnahme der höheren Pflanzen gemässigter Klimate durch oberirdische Organe. Flora **117**: 221-269.

WHITNEY, J. B. 1942. Effects of the composition of the soil atmosphere on the absorption of water by plants. Ohio State Univ., Abs. Doc. Diss. **38**: 97-103.

WIERSMA, D. and VEIHMEYER, F. J. 1954. Absence of water exudation from roots of plants grown in an atmosphere of high humidity. Soil Sci. **78**: 33-36.

WIERSUM, L. K. 1957. The relationship of the size and structural rigidity of pores to their penetration by roots. Plant & Soil **9**: 75-85.

WILLIAMS, W. T. and AMER, F. A. 1957. Transpiration from wilting leaves. Jour. Exp. Bot. **8**: 1-19.

WILSON, B. D., and RICHARDS, S. J. 1938. Capillary conduction of peat soils at different capillary tensions. Jour. Amer. Soc. Agron. **30**: 583-588.

WIND, G. P. 1955a. A field experiment concerning capillary rise of moisture in a heavy clay soil. Neth. Jour. Agr. Sci. **3**: 60-69.

————. 1955b. Flow of water through plant roots. Neth. Jour. Agr. Sci. **3**: 259-264.

ZAMFIRESCU, N. 1931. Cercetari asupra Absorptiunii apei prin organele aeriene ale plantelor. Bull. Min. Agr. Domenilor **3**: 3-6.

LIBERATION OF ORGANIC SUBSTANCES FROM HIGHER PLANTS AND THEIR ROLE IN THE SOIL SICKNESS PROBLEM[1]

HORST BÖRNER

Institut für Pflanzenschutz, Stuttgart-Hohenheim, Germany

Introduction 394
Liberation of Organic Substances from Higher Plants 396
Root Excretions 396
Excretions from Intact Roots 396
Amino Acids 396
Sugars 396
Scopoletin and Scopoletin Glycoside 398
Trans-Cinnamic Acid 399
Synthetic Growth Substances 399
Enzymes 400
Excretions from Excised Roots 400
Supplement 400
Liberation of Organic Compounds from Seeds and Fruits 401
Amino Acids and Sugars 401
Flavones 402
Phenolic Compounds 403
Gaseous Excretions (Ethylene, Ammonia, Hydrocyanic Acid) 403
Liberation of Organic Compounds from Plant Residues 403
Phenolic Compounds 404
3-Acetyl-6-methoxybenzaldehyde 404
Amino Acids 405
Amygdalin 406
Phlorizin 406
Supplement 407
Excretions from Leaves 408
Absinthin 408
Amino Acids 409
Juglone (5-Hydroxy-1,4-naphthoquinone) 409
The Role of Excreted Organic Compounds in the Interaction of Higher Plants 410
The Possibility of Direct Action of Excreted Compounds upon other Plants 410
Microbial Decomposition of Compounds Excreted by Higher Plants to Phytotoxic Substances 412
Influence of the Microbial Balance in Soil by Excreted Compounds 412
Summary and Conclusions 413
Literature Cited 414

1. Pertinent literature through August, 1958, is considered in this review. The writer herewith expresses thanks to Dr. George L. McNew, Managing Director of the Boyce Thompson Institute, Yonkers, N. Y., and to his associate, Dr. Lela V. Barton, for valuable advice and helpful criticism during the preparation of this review at the Institute under terms of a grant from the U. S. International Cooperation Administration administered by the National Academy of Sciences.

INTRODUCTION

The liberation of organic substances from higher plants is often discussed, especially in connection with the so-called soil sickness problem. The observation that toxic substances released into the soil by plant roots affected the subsequent growth of the same or other species (toxic theory) was made many years ago by De Candolle (42), Plenk (130), Daubeny (40), Uslar (164) and Liebig (88). Liebig supported this theory only in the very beginning of his scientific career. Later, as a result of his pioneer investigations on the importance of inorganic nutrition for plant growth, he came to the conclusion that inorganic materials are most important for soil productivity. Thus the idea that toxic excretions from plant roots might be responsible for declining crop yields was superseded completely.

In the beginning of this century the toxic theory again was emphasized by the investigations of Schreiner *et al.* (149-151) and of Pickering (128). These authors, however, obviously over-emphasized the importance of toxic materials to explain infertility of soils. Further investigations made before 1937 are covered in a comprehensive review by Loehwing (93) and will not be discussed here. After a critical study of the reviewed literature, Loehwing concluded that the publications up to 1937 do not indicate that excreted materials are a major factor in soil sickness (142). Early workers in the field were not able to isolate or identify the postulated toxins, nor did they demonstrate the liberation of toxins from roots. For this reason other factors, such as nutritional impoverishment of the soil, could never be excluded as possible causes of soil sickness.

The publication of Molisch's work (114) on the influence of one plant upon another and the subtitle "Allelopathy" marked a new period of work on problems of the influence of higher plants on each other. Especially the new expression "allelopathy" was introduced to botanical literature. In its original meaning this expression characterized the interactions of all plant organisms, microorganisms as well as higher plants on each other, caused by products of plant metabolism. The mutual relations between microorganisms and higher plants or between microorganisms now represent independent experimental fields. The influence of plant organisms on each other may be classified as follows:

INFLUENCE OF PLANT ORGANISMS ON EACH OTHER (66, 135)

Action of			
microorganisms upon microorganisms by antibiotics (antibiosis)	higher plants upon microorganisms by phytonzides Waksman (169)	microorganisms upon higher plants by marasmines Gäumann u. Jaag (58)	higher plants upon higher plants by allelopathica allelopathy

Grümmer (66) named the substances which are responsible for these effects on higher plants, "Koline" (=inhibition compounds), while Rademacher (135) suggested, analogously to the terms "antibiotics", "phytonzides" and "marasmines", the more extensive designation "allelopathica".

The investigations of Molisch on the liberation of ethylene from ripening fruits and the effect of this compound on the growth of higher plants (45, 50, 51) gave new impetus to work on allelopathic problems. Besides a simple description and enumeration of competitive phenomena, investigators began to search for the real causes. Special interest was devoted to chemical characterization of the compounds excreted from higher plants into soil, and to the determination of their phytotoxic activity. These investigations were aided by the introduction of improved methods of chemical identification, such as paper chromatography, so that it became possible more and more frequently to identify even small amounts of liberated compounds.

Excretion of organic substances occurs from all parts of a plant, so a review cannot be limited to root excretions only. The liberation of organic substances from seeds and fruits, leaves and, as recent experiments have shown, especially from plant remains must also be included. The present literature survey will deal mainly with papers in which isolated substances from higher plants could be identified or at least could be classified within a definite group of substances.

Comprehensive reviews concerning the entire field of allelopathy have been published by Loehwing (93; literature until 1937), Molisch (114; especially on ethylene, literature to 1937), Bonner (27; literature to 1950), Knapp (79; with special consideration to the experimental plant sociology, literature to 1954), Grümmer (66; monograph, literature to 1955), Rademacher (136; literature to 1957) and Martin (97).

The numerous publications dealing with the liberation of ethylene from plant tissues will not be discussed in this review. A detailed survey concerning these experiments is to be found in the monograph of Grümmer (66).

LIBERATION OF ORGANIC SUBSTANCES FROM HIGHER PLANTS

Within the last few years many investigators succeeded in the identification of substances liberated from higher plants by means of paper chromatography. Besides amino acids, especially sugars, nucleotides, flavones and other aromatic compounds have been detected.

ROOT EXCRETIONS

EXCRETIONS FROM INTACT ROOTS

AMINO ACIDS. As early as 1936 Virtanen *et al.* (168) reported upon the excretion of lysine and aspartic acid by root nodules of leguminous plants. It was not until 20 years later, however, that other investigators were able to detect the excretion of amino acids from other plants. The existing literature concerning the liberation of amino acids from roots is specified in Table I. It should be stressed, however, that some experiments of the publications mentioned in Table I were not carried out under strict physiological conditions. Kandler (74) used excised maize roots for his experiments, while Martin (107, 108) demonstrated root excretions only in distilled water as a growth medium. The reported results of Parkinson (125), on the other hand, indicated the possibility that detected amino acids are given off from caryopses, not by roots (15). Katznelson *et al.* (76) demonstrated a more intensified liberation of amino acids during transitory wilting of experimental plants. The experiments of Linskens and Knapp (91) demonstrated qualitative as well as quantitative differences in the liberation of amino acids, depending on whether *Trifolium repens*, *Lolium perenne* and *Artemisia absinthium* were planted together or were growing in separate cultures. Tryptophan and aminobutyric acid were detectable in mixed culture, but they could not be identified if the plants were growing separately.

SUGARS. Publications with regard to excretion of glucose and fructose from roots are entered in Table I. In this case also it should be noted that some of the reported results were not obtained under normal

TABLE I

LIBERATION OF AMINO ACIDS AND SUGARS FROM THE ROOTS OF HIGHER PLANTS

Amino acids and sugars detected	Plants*										
	I	II	III	IV	V	VI	VII	VIII	IX	X	XI
Leucine/isoleucine	+	+	+	+			+	+	+		+
Valine	+	+	+	(+)	+		+	+	+		
Aminobutyric acid	?	+		+	+						
Glutamine	+	+	+	+							+
Alanine	+	+	+	+			+	+	+	+	+
Asparagine	+	+	+	+	+	+					+
Serine	?	+	+	+	+		?	?	+		
Glutamic acid	+	+	+	+	+	+	+	+	?	+	
Aspartic acid	+	+	+	+	+	+	+	+	+	+	
Cystine and cysteine		+				+				+	
Glycine		+			+					+	
Phenylalanine	+	+		+							
Threonine	+	+		+							
Tyrosine		+		+		+				+	
Lysine		+		+		+					+
Proline					+						
Methionine				(+)		+					
Tryptophan				+		+					
Homoserine				+							
Glucose		+		+						+	
Fructose		+		+							
Reference	(55)	(77, 108, 125, 138)	(54, 74)	(77, 138, 168)		(54)	(1)	(91)	(91)	(73)	(77)

*I: *Helianthus annuus;* II: *Avena sativa;* III: *Zea mays;* IV: *Pisum sativum;* V: *Asparagus;* VI: *Oryza;* VII: *Trifolium repens;* VIII: *Lolium perenne;* IX: *Artemisia absinthium;* X: *Solanum lycopersicum;* XI: *Triticum aestivum.*

(+): trace; +: indicates presence of amino acid or sugar.

physiological conditions. Martin (107, 108) used distilled water without nutrients for his experiments, while Katznelson *et al.* (76, 77) found a remarkable excretion of sugars only if the plants were brought to transitory wilting.

A direct influence of liberated sugar on other plants can not be expected because the amount (18) and the physiological effectiveness (30, 86) of the liberated substances are too small.

SCOPOLETIN AND SCOPOLETIN GLYCOSIDE. Intensive investigations were carried out on the liberation of fluorescing compounds from oat roots. Eberhardt (47, 48) cultivated oat seedlings in distilled water and sand culture, and showed a relatively strong liberation of blue fluorescent compounds, identified by means of paper chromatography as scopoletin (7-hydroxy-6-methoxyccumarin) and a β-glycoside of the scopoletin. Another unidentified glucoside, called simply "root-tip-glycoside", was also isolated (see also 59). Of these three compounds, scopoletin seems to be dominant. When oats are germinated on moistened filter paper in a water-saturated atmosphere, small water drops form on the root hairs. Under certain conditions fluorescing substances can be detected in those drops. Eberhardt (48) questions whether the substances are genuine root excretions or are due to an irreversible semi-permeability of dead cells. He concluded, however, that scopoletin is liberated from living root hairs. Full turgescence and streaming of the plasma in 0.7M cane sugar solution served as criteria of living root hairs. Martin (107, 108) dealt chiefly with quantitative determination of the scopoletin liberated by oat plants and the influence of various conditions on the liberation. He found the excretions small under good physiological conditions but increasing in amount as growth conditions became more unfavorable. Martin (108) found the excretion of scopoletin from 100 seedling roots in nutrient solution amounted to 3.8 μg.; and in distilled water, 121.9 μg. after 86 hours.

It is probable, therefore, that the liberation of scopoletin in Eberhardt's experiments (47, 48) resulted chiefly from the unfavorable experimental conditions (distilled water). Furthermore, Martin (108) found that oxygen supply, temperature, potassium and calcium ions (94) have noticeable influence on excretion. It appears that the liberation of scopoletin can be considered a way of measuring the physiological condition of the oat root cells.

Scopoletin and related unsaturated lactones act as germination inhibitors and growth substances (60, 61, 68, 110, 155). Among recent reports, those of Avers and Goodwin (5), Goodwin and Taves (60) and Pollock *et al.* (131) are of special interest. These investigators have shown that scopoletin inhibits the root growth of oats and *Phleum pratense*, even at a concentration of 3×10^{-4}M. Also, Andrea (3) dem-

onstrated growth inhibition of pea roots when the seeds were soaked in a solution of scopoletin (50 p.p.m.). At a lower concentration (1 p.p.m.), however, he found growth promotion. In the same way Libbert and Lübke (87) demonstrated that old seeds of *Sinapis alba* were apparently inhibited by scopoletin at the incredibly low concentration of 10^{-11} g./ml.

TRANS-CINNAMIC ACID. A well-known example of the excretion of an organic compound from roots of higher plants is that of *trans*-cinnamic acid from *Parthenium argentatum* (guayule) as demonstrated by Bonner and Galston (26, 28).

In numerous experiments Bonner found that older guayule plants inhibited the growth of seedlings of the same species. It was also possible to demonstrate that water, leached through soils which contained old guayule plants, was toxic to seedlings, while water, leached through soils with young plants, did not show any toxicity. From these results one can conclude that the toxin is liberated from older plants. The isolated toxin could be identified as *trans*-cinnamic acid. This substance was highly toxic to *Parthenium* seedlings. At a concentration of 50 mg. per liter, growth of the seedlings was retarded 50 per cent in water culture, and even at 1 mg. per liter apparent growth inhibition was noted (see also 165, 167). On the other hand, young tomato seedlings were one-hundredth as sensitive to the guayule toxin. Further statements concerning the influence of cinnamic acid and related compounds upon germination are mentioned in the publication of Mayer and Evenari (111) and Börner (21). The original assumption that the double bond in the side chain is the cause of the physiological activity of cinnamic acid could not be verified in many cases (143), since hydrocinnamic acid often showed the same activity (111, 129, 145). Because cinnamic acid is decomposed in soils by microorganisms, it was not possible to demonstrate a toxicity in soils containing guayule plants for six years. *Trans*-cinnamic acid seems to have, therefore, no economic importance under natural conditions (27).

SYNTHETIC GROWTH SUBSTANCES. Treatment of the above ground parts of beans with α-methoxyphenylacetic acid and 2, 3, 6-trichlorobenzoic acid (89, 132) results in typical growth substance symptoms. Similar symptoms have been produced on untreated plants growing in the same pot with the treated ones. It is obvious that the applied compounds are excreted into the soil by the roots of the treated plants and taken up by the untreated beans.

ENZYMES. Recently, Russian investigators have found excretions of enzymes from plant roots. They demonstrated that 24 plant species excreted ten different extra-cellular enzymes, and that the occurrence of these substances is not limited to plants with mycorrhizae (cit. from Grümmer (66) p. 84). Rogers *et al.* (137) reported upon the breakdown of organic phosphorus compounds by exoenzymes of plant roots. These results may be significant, since several organic phosphorus compounds have been detected in the soil (2, 46, 181, 182). The ability of different plants to decompose these substances provides them with an additional supply of phosphorus.

EXCRETIONS FROM EXCISED ROOTS

Some investigations have been made using excised seedling roots in distilled water, salt solution or dilute acids. Lundegårdh and Stenlid (94) reported that nucleotides were excreted from excised pea and wheat roots. Fries and Forsman (56) identified adenine, guanine, uridine and cytidine liberated in sterile solutions containing excised roots of peas. In an experiment with seedling roots cut from wheat and *Arachis hypogaea* plants, Lundegårdh and Stenlid (94), as well as Petrů and Chrastil (127), found, as a root excretion, a flavone which probably corresponds to the compound 3, 4-dihydroxyflavone. Nance and Cunningham (120) reported that acetaldehyde was released from excised plant roots.

It is doubtful that any of these substances could be called root excretions in the true sense of the word, since chemicals could leach readily from the cut surface of the root into the surrounding medium.

SUPPLEMENT

Several publications in which excretions of toxic substances from roots are indicated, but in which effective toxins have not yet been identified, will be discussed briefly. Detailed experiments about the absence of therophytes and the degradation of plant societies as well as the new colonization of fallow land have been carried out by Becker *et al.* (6-9), Guyot and Massenot (67) and Deleuil (43, 44). The results obtained by these authors strongly suggest that toxic substances released into the soil from the roots of certain plants have a decisive role in the problems mentioned above. A growth reduction beginning in the center of a compact plant group, after a definite time (11, 37, 38) has also been attributed to the same causes. Though toxic compounds were often detected within the roots of the experimental plants,

the possibility that other causes, such as nutrition deficiency, may also be responsible for the growth depression has not yet been excluded (119).

Varma (166) and Hurtig (72) have reported on the toxic action of water leached from roots upon the germination and development of other plants. However, very little information about the kind of toxins was secured. *Avena sativa* considerably inhibits the growth of *Sinapis arvensis* when the plants are grown together in the same water culture (22). Because the plants were allowed to grow in separate pots but in the same circulating nutrient solution, the different growth could not be attributed to space and light competition. The amount of nutrition was currently controlled and supplied, so that a nutrition competition was not involved. From these investigations and water culture experiments concerning the self intolerance of flax (24, 25), it seems to be obvious that the growth differences are caused by substances liberated from roots (10, 71). But it should be emphasized that, as long as it is not possible to isolate and identify the active substances, the cause of depressed growth cannot be stated with certainty for other fatcors, since allelopathy may be operating.

LIBERATION OF ORGANIC COMPOUNDS FROM SEEDS AND FRUITS

A large number of publications demonstrate the occurrence and the liberation of germination-inhibiting substances from seeds and fruits. Reviews are given by Evenari (52), Moewus *et al.* (113), Toole *et al.* (162), Knapp (79) and in an extensive work by Niemann (121). However, little is known about the chemical character of these inhibiting substances. Except for the identification of some amino acids, sugars, flavones, phenolic compounds and ethylene, the investigators have given only approximate data about the kind of substance involved, i.e., tanning matters, organic acids, mustard oils, etc. (121).

AMINO ACIDS AND SUGARS. Niemann (121), in a comprehensive review prepared in 1952, summarized results which showed that the seeds and fruits of numerous plant species excrete ninhydrin-positive compounds during the swelling period. These results were confirmed and expanded by Kugler (85) and Grimm (65). Somewhat later, other authors were successful in identifying numerous liberated amino acids and sugars from swelling seeds of *Secale cereale, Triticum aesti-*

vum and *Hordeum vulgare* (see Table II and 15) as well as *Trifolium repens, Lolium perenne* and *Artemisia absinthium* (91). Linskens and

TABLE II

LIBERATION OF AMINO ACIDS AND SUGARS FROM SEEDS AND FRUITS

Amino acids and sugars detected	Plants*					
	I	II	III	IV	V	VI
Alanine	+	+	+	+	+	+
Valine		+		+	+	+
Leucine				+	+	+
Serine	+	+				
Aspartic acid	+	+	+	+	+	+
Asparagine				+	+	+
Glutamic acid	+	+	+	+	+	+
Glucose				+	+	+
Fructose				+	+	+
Xylose					+	
Reference	(91)	(91)	(91)	(15, 18)	(15, 18)	(15, 18)

*I: *Trifolium repens;* II: *Lolium perenne;* III: *Artemisia absinthium;* IV: *Secale cereale;* V: *Hordeum vulgare;* VI: *Triticum aestivum.*

+Indicates presence of amino acid or sugar.

Knapp (91) found that amino acids were liberated only from non-viable seeds.

FLAVONES. Seeds of *Trifolium repens* and *Trifolium hybridum* excrete a yellow dye within 24 hours after being placed on moist filter paper for germination (79). This excretion is noted particularly from seeds capable of germination. By paper chromatographic comparison of color reactions and absorption spectra, quercetin and myricetin could be identified as the liberation products of *Trifolium repens,* and myricetin as that of *Trifolium hybridum* (20). To establish direct proof of liberation of those compounds, the seeds were laid on the starting line of a moistened paper chromatogram and left for 60 hours in a water-saturated container. Chromatography of the yellow dyes, given off to paper, revealed the same result: myricetin was separated only from *T. hybridum;* quercetin, however, was found in both clover species. From the spot size and the color intensity it was clearly evident, however, that *T. repens* excreted considerable more myricetin than quercetin.

PHENOLIC COMPOUNDS. Phenolic substances can be detected in spots where flax *(Linum usitatissimum)* seeds have been kept on moist filter paper for 12 hours. Closer characterization of the separated substances showed that the detected chemical, in all probability, was a glycoside. After hydrolysis, the phenolic aglycon and glucose could be demonstrated by means of paper chromatography. In addition to the aglycon, Börner (21) identified ferulic acid, *p*-coumaric acid and *p*-hydroxybenzoic acid in the alkaline or acid hydrolysate. The identified substances did not inhibit the germination of seeds of 12 species. The growth in length of flax roots, on the contrary, was reduced markedly (21).

GASEOUS EXCRETIONS (ETHYLENE, AMMONIA, HYDROCYANIC ACID). Gaseous excretions, identified up to the present time, are mainly ethylene, hydrocyanic acid and ammonia. As mentioned before, ethylene liberation is discussed fully in the publications of Grümmer (66) and Molisch (114) and will, therefore, not be reviewed in this paper. Stout and Tolmann (158, 159) have reported on the liberation of ammonia from *Beta vulgaris* and other seeds. The concentration of the liberated compounds was so high that germination of *Agrostemma githago* in Petri dishes on filter paper was inhibited completely by the presence of *Beta* seeds (66, p. 39). The inhibition disappeared, however, when soil was used as the germination medium. It is apparent, therefore, that excretion of ammonia is of no importance under natural conditions.

Ullmann (163) and Grümmer (66) have shown that bitter almonds, containing amygdalin, inhibit the germination of wheat and poppy seeds in Petri dishes, while the glycoside-free sweet almonds do not have any influence. The authors assume that amygdalin is broken down by enzymes into glucose, benzaldehyde and the germination inhibitor hydrocyanic acid. Also, in this case, it is doubtful that hydrocyanic acid would have any effect under natural circumstances because this compound is either absorbed by the soil particles or released from the soil as a gas.

THE LIBERATION OF ORGANIC COMPOUNDS FROM PLANT RESIDUES

Recently it has become more and more apparent that the large quantities of plant remains (roots, leaves) yearly left in the soil are very important in the effect of higher plants on each other. In a number of investigations it has been possible to identify the substances liberated

from plant remains into the soil and to demonstrate their phytotoxic activity.

PHENOLIC COMPOUNDS. Collison (34, 35) reported as early as 1925 on the detection of inhibiting substances in wheat straw. Many years later these investigations were resumed by Winter and Schönbeck (147, 176-178). From the results of these authors it is apparent that straw as well as roots of cereals contain cold water-soluble substances which inhibit the development of various plants. The inhibiting effect on wheat roots which grew in straw or root extracts of barley, rye, wheat and oats was still visible at a concentration of straw to water of 1 to 400. The inhibiting material was also detected in water extracts of soils containing different amounts of straw and root material. After harvest the concentration of the inhibiting substances was, as it was to be expected, higher under stubble rows than between the rows. Thus it was demonstrated that phytotoxic substances were given off from cereal remains and were present in the soil under natural conditions.

Experiments on the chemical structure of inhibiting compounds have shown that cold water extracts of straw of barley, rye, wheat, and also alcoholic extracts of roots of these plants, contain ferulic acid (4-hydroxy-3-methoxycinnamic acid), *p*-coumaric acid (4-hydroxycinnamic acid), vanillic acid (4-hydroxy-3-methoxybenzoic acid) and *p*-hydroxybenzoic acid (16, 17, 18). The synthetic compounds inhibited the growth of rye and wheat roots in water culture at a concentration of 10^{-5} g./cm.^{3} (10 p.p.m.) 20 to 30 per cent.

3-ACETYL-6-METHOXYBENZALDEHYDE. The observation of Went (172) that the desert shrub *Encelia farinosa* is very rarely associated with annual plants, gave rise to the assumption that toxic substances excreted by these plants are responsible for this situation. Gray and Bonner (62) confirmed this observation. Furthermore, these authors found that from chopped leaves of *Encelia* a toxic compound is washed into the soil, which is able to inhibit corn and tomato plants as well as seedlings of species commonly associated with desert shrubs. On the other hand, the substances are almost non-toxic to *Encelia* itself, barley, oats and sunflowers. The toxic principle was later identified as 3-acetyl-6-methoxybenzaldehyde, a compound not previously found in plants (63). A concentration of 250 mg. per liter of the synthesized compound was sufficient to kill tomato plants within 24 hours. Furthermore, they demonstrated that chopped leaves retained their toxicity

for almost a year. Thus the toxic compound is washed out of leaves into the soil by rain during the entire vegetation period of the *Encelia* plant. While the experiments by Gray and Bonner strongly suggested a toxic action of the *Encelia* toxin also under natural conditions, Muller (117, 118) pointed out that the toxin is of no importance in soil in inhibiting the fruit of higher plants because the substance is adsorbed on soil particles or broken down by microorganisms. Furthermore, Muller stressed that *Franseria dumosa,* also a desert shrub, is associated very closely with other plants, although the leaves of this shrub contain toxic substances.

AMINO ACIDS. The possibility that falling foliage has an influence on the vegetation beneath trees has been mentioned by many authors. In such a way one could demonstrate (31, 175, 179) that cold water extracts of beech foliage or pine needles as well as dried cereal leaves contain substances inhibiting germination. Knapp and Linskens (80) were able to identify a number of amino acids in the dried leaves of various plants. The concentration of detected amino acids was very different in the various experiments reported (Table III). This is due

TABLE III

DETECTIONS OF AMINO ACIDS IN WATER EXTRACTS OF LEAF STRAW (ABOUT 3-MONTH-OLD) OF DIFFERENT PLANTS. Modified after Knapp and Linskens (80)

Amino acids detected	Plants*							
	I	II	III	IV	V	VI	VII	VIII
Alanine	+		+	+	(+)			+
Valine		+			+?			+
Leucine								+
Serine	+	++		++	+++	+++	+++ +	++
Cysteine	+	++	+	+++	+	++		+
Aspartic acid		+	+	+++	+	+	++	++
Glutamic acid	(+)	(+)	(+)	+++	+		(+)	(+)
Tryptophan ?	(+)	+		+	+	+	(+)	+++

*I. *Fagus silvatica;* II. *Ilex aquifolium;* III. *Carpinus betulus;* IV. *Luzula nemorosa;* V. *Deschampsia flexuosa;* VI. *Vaccinium myrtillus;* VII. *Calluna vulgaris;* VIII. *Hedera helix.*

(+), Trace; +, up to 5 μg.; ++, up to 10 μg.; +++, up to 15 μg.; ++++, up to 20 μg.

mainly to the species, not to the location of the plants. Knapp and Linskens (80) pointed out that the quantitative and qualitative differences of amino acids in plant species are high enough to have an influence on the microorganism flora of the soil and probably also to have a selective importance for higher plants.

AMYGDALIN. Peach seedlings, when replanted in the same soil, show a decrease in yield and growth. The causes are not yet completely known. Numerous experiments have been carried out to solve the problem, which is widespread in the main cultivation areas of peaches in North America (California, U.S.A., and Ontario, Canada). The researches have dealt with deficiencies of nutrients and minor elements, influence on the growth by plant pests and diseases as well as the importance of toxic substances from root residues (84, 133, 134). The results indicate that the soil sickness can not be reduced to a single factor. The entire problem seems to be a complex one, in which nematodes (115, 116), microorganisms (171) and toxic substances all may play a role.

Proebsting and Gilmore (134) have shown that addition of peach roots to healthy soil inhibits the growth of the peach seedlings very obviously, and that the root bark contains the toxic principle. Patrick (126) was able to demonstrate that roots, remaining in soil, release amygdalin which is broken down by soil microorganisms into glucose, hydrocyanic acid and benzaldehyde. Amygdalin, a natural constituent of the peach root bark, was detectable in amounts of 50 mg. per g. root bark (170). While amydalin is non-toxic to peach seedlings, the breakdown product, benzaldehyde, produced inhibition of respiration and browning of the root tips (152, 153). The toxic hydrocyanic acid probably has no effect because the compound will leave the soil as a gas. In order to determine the degree of toxicity of benzaldehyde, Patrick (126) measured the inhibition of respiration in a Warburg apparatus. This test is probably not sufficient to get a final measure of the effectiveness of the toxin under natural conditions. It is suggested that, in addition to respiration measurements, the toxicity of benzaldehyde in water and soil cultures should be tested.

PHLORIZIN. Soil sickness in fruit culture is a problem of economic importance, especially in many parts of Germany. Very often it is impossible to replant apple trees on the same soil, and growers are compelled, therefore, to plant apple seedlings in soils never before used for apples. The causes of this soil sickness have not yet been

found (29, 78). Beside an impoverishment of the soil regarding micro-elements (83) and damage to the roots by nematodes (123, 160), root residues become, as in the peach replant problem, increasingly important (53, 144). The assumption that root residues have an effect on apple replants is based on the results of Fastabend (53). He revealed that apple roots and water leached through sick soil produced the soil sickness if added to healthy soil. These results were confirmed with apple seedlings cultured in water solution containing root bark. As little as 1 g. air-dried root bark of a 16-year-old apple tree per 500 $cm.^3$ nutrient solution reduced the root and stem growth to 50 per cent within 30 days. Even 200 mg. of bark showed remarkable inhibiting effects (23).

In experiments concerning the chemical nature of the inhibiting compounds it was possible to detect phlorizin, a natural constituent of apple root bark, in water leached through soils containing apple roots and in water culture containing apple root bark. This substance, however, is broken down by microorganisms in water cultures and soils in the following way (19):

$$\text{phlorizin} \rightarrow \text{phloretin} \begin{cases} \nearrow \text{p-hydroxyhydrocinnamic acid} \rightarrow \text{p-hydroxybenzoic acid} \\ \searrow \text{phloroglucinol} \end{cases}$$

The phytotoxic effect of phlorizin and its breakdown products were proved further in water culture experiments at concentrations from 10^{-3} to 10^{-5}M. The results have shown that all identified compounds, except phloroglucinol, inhibited root growth about 15 to 25 per cent at a concentration of 10^{-4}M (23). The inhibiting effect on growth in length of stems is very different and is shown only by phlorizin and phloretin. In this case, however, we have to consider that pure phlorizin and phloretin are also broken down in nutrient solution. As a result, besides phlorizin, phloretin, phloroglucinol, *p*-hydroxyhydrocinnamic acid and *p*-hydroxybenzoic acid are present and, in all probability, caused an increased growth depression. Whether the direct action of phlorizin and its breakdown products are the main causes of apple soil sickness cannot be finally determined at this time because other factors, for instance, an influence on the microbial balance by the identified substances, have also to be taken into consideration.

SUPPLEMENT

Closely related to the apple and peach soil sickness is the "slow decline of citrus" in California. In this case also there is

a decrease in growth if citrus is replanted on soils which have previously produced citrus trees (105). Martin and his co-workers in California have been working intensively on this problem for many years. The results of their investigations have shown that citrus growth is much improved if the sick soil is treated by chemicals (CS_2, Chloropicrin, etc.) or steaming (98, 103, 104). Since these treatments have a qualitative and quantitative influence on the microorganism flora of the soil, Martin and his co-workers have assumed that a disturbed microbial balance is involved in the citrus soil sickness problem (99, 100, 102, 106). The treatment by chemicals did not improve the growth, however, to a point comparable to growth in healthy soils which never before produced citrus.

On the other hand, soil sickness can be removed completely by washing the soil with two per cent H_2SO_4 or two per cent KOH (101). This indicates that, besides microorganisms, toxic substances are involved in the citrus problem. However, no one has succeeded in identifying the effective compound or compounds.

EXCRETIONS FROM LEAVES

A large number of facts are known about the influences of higher plants on each other by excretion from leaves (66, 81). Gaseous excretions are said to be responsible for the effects, especially with medicinal and spice plants, most of which have high contents of ethereal oils. Up to the present, however, the liberated compounds have been isolated and identified in only a few cases.

ABSINTHIN. An exceptionally good example of experimentation on the influence of higher plants is the work of Bode (13) on the liberation of absinthin from leaves of *Artemisia absinthium* and its effect on other plants. Bode showed that rain washed substances out of the T-form hairs and the secretion flaps of the oil glands. These substances inhibited, strikingly, the growth of *Foeniculum vulgare* and quite a number of other plants (57). Also addition of pulverized *Artemisia* drug and fresh cut leaves to the soil resulted in inhibition and delay of germination, repression of growth and increased succulence of the leaves of *Foeniculum*. The liberated compounds which could be detected consisted of up to 80 per cent of organic substances, with the bitter principle predominating in quantity, so that this compound is believed to be the responsible inhibiting factor.

Recent investigations have revealed that absinthin belongs to the

guaianolide group (122), but its full chemical composition has not yet been determined.

TABLE IV

LIBERATION OF AMINO ACIDS FROM TOMATO LEAVES DURING DIPPING FOR 30 MINUTES IN WATER. DETECTED AMOUNT IN μg. PER 100 cm.² LEAF SURFACE. Modified after Linskens (90)

Amino acids	Liberation of amino acids under natural conditons	Liberation of amino acids after influence of lycomarasmine	Liberation of amino acids after influence of fusaric acid
Glycine	29.0	88.0	69.0
Alanine	15.4	31.8	29.8
Serine	11.8	34.8	46.2
Glutamic acid	6.7	11.0	18.4
Proline	7.4	40.7	32.0
Tyrosine	9.2	23.6	21.2
Leucine	11.4	52.8	45.5

AMINO ACIDS. Linskens (90) investigated the influence of lycomarasmine and fusaric acid on the liberation of amino acids and inorganic salts from tomato leaves. As Table IV shows, the liberation of amino acids is greatly increased by the toxins, though healthy plants also excrete measurable concentrations. No liberation of sugars could be detected.

Little is known about the activity of individual amino acids on the growth of higher plants. Audus and Quastel (4) showed that tryptophan, even at a concentration of 10 p.p.m., seems to inhibit the growth of cress. Also Grimm (65) found a strong effect of tryptophan compared to other amino acids, which act chiefly in concentrations of 10^{-3} and 10^{-4} g./cm.³ on the germination of cress. The investigations upon the physiological effectiveness of amino acids were carried out mainly in Petri dishes. In sand cultures even concentrations of 10^{-3} g./cm.³ do not have any influence (18). This means that we cannot expect an immediate influence of liberated amino acids on other plants under natural conditions.

JUGLONE (5-HYDROXY-1,4--NAPHTHOQUINONE): The toxic influence of *Juglans nigra* on other plants is a well known example of the influence of higher plants on the growth of other plants, caused in all probability by a definite compound. It has been observed by

several investigators that potatoes, tomatoes, apple trees and other plants were seriously injured or even killed when partly under or in the immediate vicinity of walnut trees (14, 36, 109, 146). Massey (109) suspected that the cause of this injury might be juglone. The experiments by Davis (41) carried out with synthetic juglone revealed that this substance is strongly toxic to alfalfa and tomato plants. However, he could not show that the compound is liberated from the walnut roots into the soil.

Juglans nigra and *Juglans cinerea* contain large quantities of hydrojuglone in the root bark as well as in the leaves and in the fruit flesh. This compound, which is not phytotoxic, is oxidized immediately on exposure to the air into toxic juglone (64).

Recent investigations by Bode (14) provided proof that effective substances, especially those influencing the growth of tomato plants, diffuse into the soil, not from the roots but from the above ground parts, particularly from the leaves of the nut trees. Water, dripping from *Juglans* trees (rain drip) as well as fallen leaves, contains these growth-inhibiting substances. The inhibiting compounds which are washed out from dead leaves are probably tannins. They have not yet been identified, but in the rain drip of nut trees Bode (14) could demonstrate, by means of different color reactions, that the toxic principle was juglone.

THE ROLE OF EXCRETED ORGANIC COMPOUNDS IN THE INTERACTION OF HIGHER PLANTS

In the classical experiments on the role of toxic root excretions in the soil sickness problem, workers early arrived at the conclusion that the excreted substances have a direct effect. More recently, however, investigators have shown that microorganisms also may play an important role. The action of these organisms results either from decomposition of the excreted compounds to phytotoxic substances or by a direct influence, possibly by a disarrangement of the microbial balance as a consequence of promotion or inhibition of different microorganisms.

THE POSSIBILITY OF DIRECT ACTION OF EXCRETED COMPOUNDS UPON OTHER PLANTS

A direct action of excreted compounds upon other plants depends first of all on the concentration, the stability and the physiological ac-

tivity of the substances released into the soil. In connection with the concentration, the question is often raised as to whether or not active secretion of organic compounds takes place in living roots. Only by an active excretion can the concentration become high enough to produce a direct effect in spite of microbial decomposition and adsorption by soil particles. The occurrence of small drops on the top of the root hairs of Gramineae in a water-saturated room was taken for a long time as proof of active liquid root excretion (39, 47). More recently, researches have shown, however, that the small drops are precipitations from the water-saturated atmosphere (48, 49) and not a pressing out of liquid from the root hair.

On the other hand, the possibility of a passive liberation of organic compounds from the intact roots must be considered. Martin (108) pointed out that passive excretion depends widely on the experimental condition. Under favorable circumstances the excretion is very low (3.2 μg. scopoletin per 100 seedlings); however, the excretion increased when the condition became more and more unfavorable and attained a maximum peak during temporary wilting of the experimental plants.

It has been noticed further that, during the entire vegetative period, small amounts of root substances are released into the soil as a permanent phenomenon of root growth by dying of root hair, cortical and calyptra cells. None of the papers mentioned above has given evidence of active root excretion. Also, the concentrations of the substances given off by passive excretion under normal conditions are so low, it is unlikely that root excretions have any extended direct toxic influence upon higher plants. Also extreme, unphysiological conditions, such as wilting of the plants, will occur under natural conditions only temporarily and lead therefore to an accumulation of liberated compounds for only a short time. None of the workers who identified substances excreted by roots of higher plants was able to demonstrate, therefore, important effects under natural conditions. Furthermore, the identified compounds, scopoletin and *trans*-cinnamic acid, which are most active physiologically, are broken down by microorganisms very rapidly (26, 49) and rendered inactive.

In the same way the excretion of organic compounds from seeds and fruits seems to be of no importance in connection with a direct influence of the higher plants on other plants, since the excretion occurs only during the germination period and the concentration of the

identified substances is not high enough to be active under natural conditions (18). On the contrary, the leaf excretions by *Artemisia* (13) and *Juglans* (14) and the liberation of organic compounds from plant remains are excellent examples of a direct action upon plants of the same or other species. Especially the investigations on the effectiveness of cereal straw and stubble (147, 178) as well as the reports on peach and apple soil sickness (23, 126) have shown that, under natural conditions, a continuous action of the inhibiting compounds is to be expected because the effective compounds will be delivered subsequently from the large quantity of the residues left in the soil in spite of detoxification by microbial decomposition over a long period.

MICROBIAL DECOMPOSITION OF COMPOUNDS EXCRETED BY HIGHER PLANTS TO PHYTOTOXIC SUBSTANCES

The results of Patrick (126) gave rise to the assumption that, besides a direct action, there is still the possibility that excreted, non-toxic compounds may be decomposed or transferred by microorganisms to phytotoxic ones. Amygdalin, a natural constituent of peach root bark, is non-toxic on peach seedlings, while the breakdown product, benzaldehyde, shows a considerable toxic effect. Similar results were obtained by Börner (23) on apple. Also in this case the microbial decomposition products of phlorizin are effective compounds. Judging from these results it seems to be possible that, besides the excreted compounds, microorganisms able to break down these substances must be present in order to produce the symptoms of soil sickness.

INFLUENCE OF THE MICROBIAL BALANCE IN SOIL BY EXCRETED COMPOUNDS

Since the detection of a rhizosphere flora by Hiltner (70), there is no doubt of the occurrence of mutual relations between higher plants and microorganisms (75). It is very likely that this strong enrichment of the microorganisms in the root and seed sphere is caused by excretions of organic compounds. For the soil sickness problem the question of importance is whether the liberated substances influence or change the microorganism flora in a specific manner, and, furthermore, to what extent a disturbed microbial balance has an influence upon other plants.

The suppression of *Azotobacter chroococcum* in the rhizosphere of different plants (112, 154), the direct detection of an inhibitor of a single fungus by the root-tip glycoside of *Avena* (148), and the

distinct reaction of the rhizosphere microorganisms to amino acids (92, 139, 140, 173) strongly suggest that the plants have a more or less specific influence upon the microorganisms in their root surroundings (18, 75). Also Rümker (141) was able to demonstrate that the behavior of a phytopathogenic fungus in the neighborhood of young seedlings was influenced, especially at the beginning of growth, by the excretions from the seeds. It is already apparent that even the small amount of substances excreted by plant roots, seeds and fruits may have an influence on the rhizosphere and spermatosphere flora, and it is to be expected that the action upon the microorganisms by substances released from plant residues is much more effective. Particularly with monocultures, large amounts of residue remain in the soil after harvest, as, for example, root, stubble and straw after harvest of cereals. If these substances have anti-microbial properties (12, 32, 33, 69, 95, 96, 124, 180), a displacement of the microbial balance can be presumed. As Winter (174) reported, addition of five per cent of leaves of *Allium porrum* to peat soil was sufficient to transform the microorganism flora completely within two weeks. Also Knösel (82) found that *p*-hydroxybenzoic acid liberated from harvest residues (18) was able, even in small concentrations, to reduce growth of actinomycetes and autochtonous bacteria while growth of zymogenous bacteria was promoted.

The exact influence of a disturbed microbial balance on higher plants can not be decided at present because investigations and results are not yet complete enough. Some works were successful in showing that culture filtrates of fungi and bacteria influence the growth of the tested plants to some extent even when very greatly diluted (156, 157, 161). Another fact, which confirms the assumption of the participation of microorganisms in soil sickness, is that toxicity can be removed by steaming soil or treating with chemicals, procedures primarily affecting all microorganisms (53, 103, 105, 144).

SUMMARY AND CONCLUSIONS

The phenomenon of allelopathy has not been fully investigated. The substances liberated into the soil by higher plants in some cases influence the growth of nearby plants and subsequent crops, but the chemical identity of the materials has been determined in relatively few instances. Enough knowledge has been accumulated, however, to suggest the following conclusions:

a) Massive secretion of organic materials from intact uninjured roots probably does not occur in most species of plants. Such materials as do diffuse from roots probably are restricted to the immediate rhizosphere and do not exercise a direct influence on other plants. These materials do affect the microorganisms of the rhizosphere and may indirectly affect the health and nutrition of the plants.

b) Substances probably are not liberated from seeds and fruits under natural conditions in sufficient quantity to affect other plants. Seed are known to stimulate or suppress microorganisms in nearby soil by releasing organic materials during germination.

c) Materials toxic to other plants may be washed into the soil from leaves and stems of *Artemisia absinthium* and *Juglans nigra* by rainfall.

d) Crop residues may be largely responsible for rendering soils toxic to subsequent crops because they are available in such large amounts that they add substantial quantities of chemicals to the soil. Such materials and their decomposition products have been isolated, identified and shown to be toxic to plants growing in soil. These materials can affect soil microorganisms either by serving as a food source or as growth inhibitors.

This brief review reveals the need for much more research upon the organic compounds secreted from roots, washed from the leaves or added to soils in crop residue. They should be identified, and their physiological effects upon various crops by either direct toxicity or indirect influence of the balance between soil microorganisms should be determined. The mere presence of a chemical in the soil cannot be accepted as evidence of toxicity until data are available on its concentration, persistence, and the nature of its decomposition products.

The over-all growth of a plant undoubtedly is determined more by the proper balance of growth factors (space, light, water, mineral nutrients, etc.) than by allelopathic factors. The combined effects of soil microorganisms and the physical forces in the environment often serve to distract attention from the effect of soil toxins; but there is enough evidence to be certain that some soils are made poisonous to plants by organic compounds from preceding crops.

LITERATURE CITED

1. Andal R., K. Bhuvaneswari, and N. S. Subba-Rao. Root exudates of paddy. Nature **178**: 1063. 1956.
2. Anderson, G. Nucleic acid derivatives in soils. Nature **180**: 287-288. 1957.

3. ANDREAE, W. A. Effect of scopoletin on indoleacetic acid metabolism. Nature **170**: 83-84. 1952.

4. AUDUS, L. J., and J. H. QUASTEL. Toxic effects of amino-acids and amines on seedling growth. Nature **160**: 222-223. 1947.

5. AVERS, CH. J., and R. H. GOODWIN. Studies on roots. IV. Effects of coumarin and scopoletin on the standard root-growth pattern of *Phleum pratense*. Amer. Jour. Bot. **43**: 612-620. 1956.

6. BECKER, Y., L. GUYOT, M. MASSENOT, et J. MONTEGUT. Sur la présence d'excrétats radiculaires toxiques dans le sol de la pelouse herbeuse a Brachypodium pinnatum du Nord de la France. Compt. Rend. Acad. Sci. [Paris] **231**: 165-167. 1950.

7. ————, ————, et J. MONTEGUT. Sur quelques incidences phytosociologiques du problème des excrétions racinaires. *Ibidem*. **232**: 2472-2474. 1951.

8. ————, et ————. Sur les toxines racinaires des sols incultes. *Ibidem*. **232**: 105-107. 1951.

9. ————, et ————. Sur une particularité fonctionelle des exsudates racinaires de certains vegetaux. *Ibidem*. **232**: 1585-1587. 1951.

10. BECQUEREL, P., et J. ROUSSEAU. Sécrétions par les racines du lin d'une substance spécifique toxique pour une nouvelle culture de cette plante. Compt. Rend. Acad. Sci. [Paris] **213**: 1028-1030. 1941.

11. BENEDICT, H. M. The inhibitory effect of dead roots on the growth of brome grass. Jour. Amer. Soc. Agron. **33**: 1108-1109. 1941.

12. BISHOP, C. J., and R. E. MACDONALD. A survey of higher plants for antibacterial substances. Canad. Jour. Bot. **29**: 260-269. 1951.

13. BODE, H. R. Über die Blattausscheidungen des Wermuts und ihre Wirkung auf andere Pflanzen. Planta **30**: 567-589. 1939.

14. ————. Beiträge zur Kenntnis allelopathischer Erscheinungen bei einigen Juglandaceen. Planta **51**: 440-480. 1958.

15. BÖRNER, H. Die Ausscheidung organischer Verbindungen aus den Samen von Roggen (*Secale cereale* L.), Weizen (*Triticum aestivum* L.) und Gerste (*Hordeum vulgare* L.) während der Quellung. Naturwiss. **42**: 48. 1955.

16. ————. Untersuchungen über phenolische Verbindungen aus Getreidestroh und Getreiderückständen. Naturwiss. **42**: 583-584. 1955.

17. ————. Der papierchromatographische Nachweis von Ferulasäure in wässrigen Extrakten von Getreidestroh und Getreiderückständen. Naturwiss. **43**: 129-130. 1956.

18. ————. Die Abgabe organischer Verbindungen aus den Karyopsen, Wurzeln und Ernterückständen von Roggen (*Secale cereale* L.), Weizen (*Triticum aestivum* L.) und Gerste (*Hordeum vulgare* L.) und ihre Bedeutung bei der gegenseitigen Beeinflussung der höheren Pflanzen. Beitr. Biol. Pflanz. **33**: 33-83. 1956.

19. ————. Untersuchungen über den Abbau von Phlorizin im Boden. Ein Beitrag zum Problem der Bodenmüdigkeit bei Obstgehölzen. Naturwiss. **45**: 138-139. 1958.

20. ————. Die Abgabe von Flavonen aus keimenden Samen von *Trifolium repens* und *Trifolium hybridum*. [Unpub.]

21. ————. Nachweis phenolischer Verbindungen in Leinsamen und ihre Abgabe während der Quellung. Flora **145**: 479-496. 1958.

22. ————. Experimentelle Untersuchungen zum Problem der gegenseitigen Beeinflussung von Kulturpflanzen und Unkräutern. Biol. Zentrbl. **77**: 310-328. 1958.

23. ————. The apple replant problem. I. The excretion of phlorizin from apple root residues and its role in the soil sickness problem. Contr. Boyce Thompson Inst. **20**: 39-56. 1959.

24. ————, und B. Rademacher. Untersuchungen zum Problem der echten Selbstunverträglichkeit des Leins (*Linum usitatissimum* L.) Zeits. Pflanzenernähr. Düng. u. Bodenk. **76**: 123-132. 1957.

25. ———— und ————. Experimentelle Untersuchungen zum Problem der Selbstverträglichkeit der höheren Pflanzen. Naturwiss. **44**: 498-499. 1957.

26. Bonner, J. Further investigation of toxic substances which arise from guayule plants: relation of toxic substances to the growth of guayule in soil. Bot. Gaz. **107**: 343-351. 1946.

27. ————. The role of toxic substances in the interactions of higher plants. Bot. Rev. **16**: 51-65. 1950.

28. ———— and A. W. Galston. Toxic substances from the culture media of guayule which may inhibit growth. Bot. Gaz. **106**: 185-198. 1944.

29. Bronsart, H. v. Der heutige Stand unseres Wissens um die Bodenmüdigkeit. Zeits. Pflanzenernähr. Düng. u. Bodenk. **45**: 166-193. 1949.

30. Brown, R., E. Robinson, and A. W. Johnson. The effects of D-xyloketose and certain root exudates in extension growth. Proc. Roy. Soc. London. B. **136**: 577-591. 1950.

31. Bublitz, W. Über die keimhemmende Wirkung der Fichtenstreu. Naturwiss. **40**: 275-276. 1953.

32. Bukatsch, F. Zur Analyse der Bakterienhemmstoffe aus den Wurzeln von Schöllkraut und ähnlichen Pflanzen. Arch. Mikrobiol. **24**: 281-296. 1956.

33. Carlson, H. J., and H. G. Douglas. Screening methods for determining antibiotic activity of higher plants. Jour. Bact. **55**: 235-240. 1948.

34. Collison, R. C. The presence of certain organic compounds in plants and their relation to the growth of other plants. Jour. Amer. Soc. Agron. **17**: 58-68. 1925.

35. ————, and H. J. Conn. The effect of straw on plant growth. 35 pp. New York [Geneva] Agr. Exp. Sta., Tech. Bull. 114. 1925.

36. Cook, M. T. Wilting caused by walnut trees. Phytopath. **11**: 346. 1921.

37. Cooper, W. S. and A. D. Stoesz. The subterranean organs of *Helianthus scaberrimus*. Bull. Torrey Bot. Club **58**: 67-72. 1931.

38. Curtis, J. T., and G. Cottam. Antibiotic and autotoxic effects in prairie sunflower. Bull. Torrey Bot. Club **77**: 187-191. 1950.

39. Czapek, F. Zur Lehre von den Wurzelausscheidungen. Jahrb. Wiss. Bot. **29**: 321-390. 1896.

40. Daubeny, C. Memoir on the rotation on crops and on the quantity of inorganic matters abstracted from the soil by various plants under

different circumstances. Phil. Trans. Roy. Soc. London, B. **135**: 179-252. 1845.

41. Davis, E. F. The toxic principle of *Juglans nigra* as identified with synthetic juglone, and its toxic effects on tomato and alfalfa plants. Amer. Jour. Bot. **15**: 620. 1928.
42. DeCandolle, A. P. Physiologie vegetale. T. III, p. 1474. 1832.
43. Deleuil, G. Mise en évidence de substances toxiques pour les thérophytes dans les associations du Rosmarino-Ericion. Compt. Rend. Acad. Sci. [Paris] **230**: 1362-1364. 1950.
44. ————. Origine des substances toxiques du sol des associations sans thérophytes du Rosmarino—Ericion. *Ibidem.* **232**: 2038-2039. 1951.
45. Denny, F. E., and L. P. Miller. Production of ethylene by plant tissue as indicated by the epinastic response of leaves. Contr. Boyce Thompson Inst. **7**: 97-102. 1935.
46. Dyer, W. J., C. L. Wrenshall, and G. R. Smith. The isolation of phytin from soil. Science **91**: 319-320. 1940.
47. Eberhardt, F. Ausscheidung einer organischen Verbindung aus den Wurzeln des Hafers (*Avena sativa* L.). Naturwiss. **41**: 259. 1954.
48. ————. Über fluoreszierende Verbindungen in der Wurzel des Hafers. Ein Beitrag zum Problem der Wurzelausscheidungen. Zeits. Bot. **43**: 405-422. 1955.
49. Eberhardt, F., und P. Martin. Das Problem der Wurzelausscheidungen und seine Bedeutung für die gegenseitige Beeinflussung höherer Pflanzen. Zeits. Pflanzenkrankh. u. Pflanzenschutz **64**: 193-205. 1957.
50. Elmer, O. H. Growth inhibition of potato sprouts by the volatile products of apples. Science **75**: 193. 1932.
51. ————. Growth inhibition in the potato caused by a gas emanating from apples. Jour. Agr. Res. **52**: 609-626. 1936.
52. Evenari, M. Germination inhibitors. Bot. Rev. **15**: 153-194. 1949.
53. Fastabend, H. Über die Ursachen der Bodenmüdigkeit in Obstbaumschulen. Landwirtschaft-Angewandte Wissenschaft, Sonderheft Gartenbau IV. 95 pp. Landwirtschaftsverlag Hiltrup/Münster, 1955.
54. Frank, H. Über den Stickstoffverlust bei alternden Pflanzen. Planta **44**: 319-340. 1954.
55. Frenzel, B. Zur Abgabe von Aminosäuren und Amiden an das Nährmedium durch die Wurzeln von *Helianthus annuus* L. Planta **49**: 210-234. 1957.
56. Fries, N., and B. Forsman. Quantitative determination of certain nucleic acid derivatives in pea root exudate. Physiol. Plant. **4**: 410-420. 1951.
57. Funke, G. L. The influence of *Artemisia absinthium* on neighbouring plants. Blumea **5**: 281-293. 1943. [after Grümmer (66)]
58. Gäumann, E., und O. Jaag. Über das Problem der Welkekrankheiten bei Pflanzen. Experientia **2**: 215-220. 1946.
59. Goodwin, R. H., and B. M. Pollock. Studies on roots. I. Properties and distribution of fluorescent constituents in *Avena* roots. Amer. Jour. Bot. **41**: 516-520. 1954.
60. Goodwin, R. H., and C. Taves. The effect of coumarin derivatives on the growth of *Avena* roots. Amer. Jour. Bot. **37**: 224-231. 1950.

61. GRAILLOT, G. [Action of some lactones related to coumarin on germination of chicory seeds. Spectral study of the compounds.] Bull. Soc. Chim. Biol. **33**: 1584-1590. 1951. Chem. Abstr. **46**: 9169c. 1952.

62. GRAY, R., and J. BONNER. An inhibitor of plant growth from the leaves of *Encelia farinosa*. Amer. Jour. Bot. **35**: 52-57. 1948.

63. ———— and ————. Structure determination and synthesis of a plant growth inhibitor, 3-acetyl-6-methoxybenzaldehyde, found in the leaves of *Encelia farinosa*. Jour. Amer. Chem. Soc. **70**: 1249-1253. 1948.

64. GRIES, G. A. Juglone—the active agent in walnut toxicity. North. Nut Grow. Assoc., Ann. Rep. **34**: 52-55. 1943.

65. GRIMM, H. Zur Physiologie und mikrobiologischen Beeinflussung genuiner Hemmstoffe von *Digitalis purpurea* L. Zeits. Bot. **41**: 405-444. 1953.

66. GRÜMMER, G. Die gegenseitige Beeinflussung höherer Pflanzen, Allelopathie. 162 pp. Gustav Fischer Verlag. Jena, 1955.

67. GUYOT, L., et M. MASSENOT. Sur la persistance prolongée de semences dormantes dans le sol de la pelouse herbeuse à *Brachypodium pinnatum* du Nord de la France. Compt. Rend. Acad. Sci. [Paris] **230**: 1894-1896. 1950.

68. HAMMER, C. L., H. M. SELL, W. KLOMPARENS, and J. R. VAUGHN. Selective inhibition of the growth of green plants and fungi by beta methyl umbelliferone. Bot. Gaz. **112**: 135-137. 1951.

69. HAYES, L. E. Survey of higher plants for presence of antibacterial substances. Bot. Gaz. **108**: 408-414. 1947.

70. HILTNER, L. Über neuere Erfahrungen und Probleme auf dem Gebiete der Bodenbakteriologie unter besonderer Berücksichtigung der Gründüngung und Brache. Arb. Deut. Landw. Ges. **98**: 59-78. 1904.

71. HIRAYOSHI, I., S. KURODA, and K. NISHIKAWA. Growth-inhibiting substances excreted by plants. Preliminary report. Inhibition of seed germination and seedling growth of Petsai [*Brassica pekinensis*] by the extract of the residue solutions of water culture. Agr. & Hort. [Tokyo] [In Japanese] **30**: 453-454. 1955. Biol. Abstr. **31**: 39559. 1957.

72. HURTIG, I. Über die allelopathische Beeinflussung der Keimfähigkeit und Triebkraft von Samen verschiedener Kulturpflanzen und Unkräuter. Wiss. Zeits. Univ. Rostock **2**: 145-157. 1953.

73. KALYANASUNDARAM, R. Production of fusaric acid by *Fusarium lycopersici* Sacc. in the rhizosphere of tomato plants. Phytopathol. Zeits **32**: 25-34. 1958.

74. KANDLER, O. Papierchromatographischer Nachweis der Aminosäurenausscheidung in vitro kultivierter Maiswurzeln. Zeits. Naturforsch. **6b**: 437-445. 1951.

75. KATZNELSON, H., A. G. LOCHHEAD, and M. I. TIMONIN. Soil microorganisms and the rhizosphere. Bot. Rev. **14**: 543-587. 1948.

76. ————, J. W. ROUATT, and T. M. B. PAYNE. Liberation of amino-acids by plant roots in relation to desiccation. Nature **174**: 1110-1111. 1954.

77. ————, ————, and ————. The liberation of amino acids and reducing compounds by plant roots. Plant & Soil **7**: 35-48. 1955.

78. KLAUS, H. Das Problem der Bodenmüdigkeit unter Berücksichtigung des Obstbaues. Landwirtsch. Jahrb. **89**: 413-459. 1939.

79. KNAPP, R. Experimentelle Soziologie der höheren Pflanzen. 202 pp. Eugen Ulmer Verlag, Stuttgart, 1954.

80. ———— und H. F. LINSKENS. Über Aminosäuren aus der Blattstreu einiger Pflanzenarten von Wäldern auf verschiedenen Böden. Naturwiss. **41**: 480-481. 1954.

81. ————, und P. Thyssen. Untersuchungen über die gegenseitige Beeinflussung von Heilpflanzen in Mischkulturen. Ber. Deut. Bot. Ges. **65**: 60-70. 1952.

82. KNÖSEL, D. Über die Wirkung aus Pflanzenresten freiwerdender phenolischer Substanzen auf Mikroorganismen des Bodens. I. Der Einfluss von *p*-Oxybenzoesäure auf die Entwicklung von Pilzen, Actinomyceten und Bakterien. Zeits. Pflanzenernähr. Düng. u. Bodenk. **80**: 225-237. 1958.

83. KOBERNUSS, E. C. Zur Ursache und Behebung der Bodenmüdigkeit bei Obstgehölzen. Kühn-Arch. **63**: 296-300. 1950.

84. KOCH, L. W. The peach replant problem in Ontario. I. Symptomatology and distribution. Canad. Jour. Bot. **33**: 450-460. 1955.

85. KUGLER, I. Zur Frage der Abgabe keimungshemmender Stoffe durch Samen. Beitr. Biol. Pflanz. **31**: 313-332. 1955.

86. LEE, A. E. The influence of various sugars on the growth in culture of intact seedlings and isolated seedling organs. Amer. Jour. Bot. **37**: 528-533. 1950.

87. LIBBERT, E., und H. LÜBKE. Physiologische Wirkungen des Scopotetins. I. Der Einfluss des Scopoletins auf die Samenkeimung. Flora **145**: 256-263. 1957.

88. LIEBIG, J. v. Complete works on chemistry. 1852. [after Bonner (27)]

89. LINDER, P. J., J. C. CRAIG, F. E. COOPER, and J. W. MITCHELL. Translocation of growth regulators, movement of 2,3,6-trichlorobenzoic acid from one plant to another through their root systems. Jour. Agr. & Food Chem. **6**: 356-357. 1958.

90. LINSKENS, H. F. Der Einfluss der toxigenen Welke auf die Blattausscheidungen der Tomatenpflanze. Phytopathol. Zeits. **23**: 89-106. 1955.

91. ————, und R. KNAPP. Über die Ausscheidung von Aminosäuren in reinen und gemischten Beständen verschiedener Pflanzenarten. Planta **45**: 106-117. 1955.

92. LOCHHEAD, A. G., and R. H. THEXTON. Qualitative studies of soil microorganisms. VII. The "rhizosphere effect" in relation to the amino acid nutrition of bacteria. Canad. Jour. Res. C. **25**: 20-26. 1947.

93. LOEHWING, W. F. Root interactions of plants. Bot. Rev. **3**: 195-239. 1937.

94. LUNDEGÅRDH, H., and G. STENLID. On the exudation of nucleotides and flavanone from living roots. Arkiv. Bot. **31A** (10): 1-27. 1944.

95. MACDONALD, R. E., and C. J. BISHOP. Phloretin: An antibacterial substance obtained from apple leaves. Canad. Jour. Bot. **30**: 486-489. 1952.

96. ———— and ————. A further survey of plants for antibacterial substances. Canad. Jour. Bot. **31**: 123-131. 1953.

97. MARTIN, H. Chemical aspects of ecology in relation to agriculture. 96 pp. Sci. Serv. Dept. Agr., Ottawa, Canada. 1957.

98. MARTIN, J. P. Effect of fumigation, fertilisation, and various other soil

treatments on growth of orange seedlings in old citrus soils. Soil Sci. **66**: 273-288. 1948.

99. ————. Fungus flora of some California soils in relation to slow decline of citrus trees. Soil Sci. Soc. Amer., Proc. **12**: 209-214. 1948.

100. ————. Effects of fumigation and other soil treatments in the greenhouse on the fungus population of old citrus soil. Soil Sci. **69**: 107-122. 1950.

101. ————. Effect of various leaching treatments on growth of orange seedlings in old citrus soil. Soil Sci. **69**: 433-442. 1950.

102. ————. Effect of soil fungi on germination of sweet orange seeds and development of the young seedlings. Soil Sci. Soc. Amer. Proc. **14**: 184-188. 1950.

103. ————, D. G. ALDRICH, W. S. MURPHY, and G. R. BRADFORD. Effect of soil fumigation on growth and chemical composition of citrus plants. Soil Sci. **75**: 137-151. 1953.

104. ————, R. C. BAINES, and J. O. ERVIN. Influence of soil fumigation for citrus replants on the fungus population of the soil. Soil Sci. Soc. Amer., Proc. **21**: 163-166. 1957.

105. ————, and J. O. ERVIN. Greenhouse studies on influence of other crops and of organic materials on growth of orange seedlings in old citrus soils. Soil Sci. **85**: 141-147. 1958.

106. ————, L. J. KLOTZ, T. A. DEWOLFE, and J. O. ERVIN. Influence of some common soil fungi on growth of citrus seedlings. Soil Sci. **81**: 259-267. 1956.

107. MARTIN, P. Qualitative und quantitative Untersuchungen über die Ausscheidung organischer Verbindungen aus den Keimwurzeln des Hafers (*Avena sativa* L.). Naturwiss. **43**: 227-228. 1956.

108. ————. Die Abgabe von organischen Verbindungen, insbesondere von Scopoletin, aus den Keimwurzeln des Hafers. Zeits. Bot. **45**: 475-506. 1957.

109. MASSEY, A. B. Antagonism of the walnuts (*Juglans nigra* and *J. cinerea* L.) in certain plant associations. Phytopath. **15**: 773-784. 1925.

110. MAYER, A. M., and M. EVENARI. The relation between the structure of coumarin and its derivatives, and their activity as germination inhibitors. Jour. Exp. Bot. **3**: 246-252. 1952.

111. ———— and ————. The activity of organic acids as germination inhibitors and its relation to pH. Jour. Exp. Bot. **4**: 257-263. 1953.

112. METZ, H. Untersuchungen über die Rhizosphäre. Arch. Mikrobiol. **23**: 297-326. 1955.

113. MOEWUS, F., L. MOEWUS, und E. SCHADER. Vorkommen und Bedeutung von Blastokolinen in fleischigen Früchten. Zeits. Naturforsch. **6b**: 261-270. 1951.

114. MOLISCH, H. Der Einfluss einer Pflanze auf die andere—Allelopathie. 106 pp. Gustav Fischer Verlag, Jena, 1937.

115. MOUNTAIN, W. B., and H. R. BOYCE. The peach replant problem in Ontario. V. The relation of parasitic nematodes to regional differences in severity of peach replant failure. Canad. Jour. Bot. **36**: 125-134. 1958.

116. ———— and ————. The peach replant problem in Ontario. VI. The relation of *Pratylenchus penetrans* to the growth of young peach trees. Canad. Jour. Bot. **36**: 135-151. 1958.

117. MULLER, C. H. The association of desert annuals with shrubs. Amer. Jour. Bot. **40**: 53-60. 1953.

118. MULLER, W. H., and C. H. MULLER. Association patterns involving desert plants that contain toxic products. Amer. Jour. Bot. **43**: 354-361. 1956.

119. MYERS, H. E., and K. L. ANDERSON. Bromegrass toxicity vs. nitrogen starvation. Jour. Amer. Soc. Agron. **34**: 770-773. 1942.

120. NANCE, J. F., and L. W. CUNNINGHAM. Evaluation of acetaldehyde by excised wheat roots in solution of nitrate and nitrite salts. Amer. Jour. Bot. **38**: 604-609. 1951.

121. NIEMANN, E. Vergleichende Untersuchungen über die Ausscheidung keimungshemmender Stoffe aus Früchten und Samen unter besonderer Berücksichtigung von *Foeniculum vulgare* Miller. Flora **139**: 185-242. 1952.

122. NOVOTNY, L., V. HEROUT, and F. SORM. A contribution to the structure of absinthin and anabsinthin. Chem. & Ind. [London] **1958**: 465-466.

123. OOSTENBRINK, M. Bodenmüdigkeit und Nematoden. Zeits. Pflanzenkrankh. u. Pflanzenschutz **62**: 337-346. 1955.

124. OSBORN, E. M. On the occurrence of antibacterial substances in green plants. Brit. Jour. Exp. Path. **24**: 227-231. 1943.

125. PARKINSON, D. Liberation of amino-acids by oat seedlings. Nature **176**: 35-36. 1955.

126. PATRICK, Z. A. The peach replant problem in Ontario. II. Toxic substances from microbial decomposition products of peach root residues. Canad. Jour. Bot. **33**: 461-486. 1955.

127. PETRŮ, E., and J. CHRASTIL. The exosmosis of flavanone from root explantations of *Arachis hypogaea* L. Folia Biol. **1**: 310-312. 1955.

128. PICKERING, S. The effect of one plant on another. Ann. Bot. [London] **31**: 181-187. 1917.

129. PLAISTED, P. H. Suppression of tomato plant terminal growth by α-cyanocinnamic acids and related compounds. Contr. Boyce Thompson Inst. **18**: 231-242. 1955.

130. PLENK, J. Physiologie und Pathologie der Pflanzen. Wien, 1795.

131. POLLOCK, B. M., R. H. GOODWIN, and S. GREENE. Studies on roots. II. Effects of coumarin, scopoletin and other substances on growth. Amer. Jour. Bot. **41**: 521-529. 1954.

132. PRESTON, W. H., JR., J. W. MITCHELL, and W. REEVE. Movement of alpha-methoxyphenylacetic acid from one plant to another through their root systems. Science **119**: 437-438. 1954.

133. PROEBSTING, E. L. A case history of a "peach replant" situation. Proc. Amer. Soc. Hort. Sci. **56**: 46-48. 1950.

134. ————, and A. E. GILMORE. The relation of peach root toxicity to the re-establishing of peach orchards. Proc. Amer. Soc. Hort. Sci. **38**: 21-26. 1941.

135. RADEMACHER, B. Die Bedeutung allelopathischer Erscheinungen in der

Pflanzenpathologie. Zeits. Pflanzenkrankh. u. Pflanzenschutz **64**: 427-439. 1957.

136. ————. Gegenseitige Beeinflussung höherer Pflanzen. Handbuch Pflanzenphysiologie **11**: 655-706, Berlin-Göttingen-Heidelberg, Springer Verlag. 1959.

137. ROGERS, H. T., R. W. PEARSON, and W. H. PIERRE. Absorption of organic phosphorus by corn and tomato plants and the mineralizing action of exoenzyme systems of growing roots. Proc. Soil. Sci. Soc. Amer. **5**: 285-291. 1940.

138. ROVIRA, A. D. Plant root excretions in relation to the rhizosphere effect. I. The nature of root exudate from oats and peas. Plant & Soil **7**: 178-194. 1956.

139. ————. Plant root excretions in relation to the rhizosphere effect. II. A study of the properties of root exudate and its effect on the growth of microorganisms isolated from the rhizosphere and control soil. Plant & Soil **7**: 195-208. 1956.

140. ————. Plant root excretions in relation to the rhizosphere effect. III. The effect of root exudate on the numbers and activity of microorganisms in soil. Plant & Soil **7**: 209-217. 1956.

141. RÜMKER, R. v. Über die Ökologie von *Ascochyta pinodella* and *Fusarium culmorum* in der Rhizosphäre anfälliger und nicht anfälliger Pflanzen. Phytopath. Zeits. **18**: 55-100. 1952.

142. RUSSELL, E. J. Soil conditions and plant growth. 636 pp. 6th ed. Longmans, Green and Co., London. 1932.

143. SAN ANTONIO, J. P. The role of coumarin in the growth of roots of *Melilotus alba*. Bot Gaz. **114**: 79-95. 1952.

144. SCHANDER, H. Die Bodenmüdigkeit bei Obstgehölzen. 66 pp. Bayrischer Landwirtschaftsverlag, Bonn-München-Wien, 1956.

145. SCHMID, H. Notiz über keimungshemmende Stoffe. Helv. Chim. Acta **27**: 1197-1199. 1944.

146. SCHNEIDERHAN, F. J. The black walnut (*Juglans nigra* L.) as a cause of death of apple trees. Phytopath. **17**: 529-540. 1927.

147. SCHÖNBECK, F. Untersuchungen über Vorkommen und Bedeutung von Hemmstoffen in Getreiderückständen innerhalb der Fruchtfolge. Zeits. Pflanzenkrankh. u. Pflanzenschutz **63**: 513-545. 1956.

148. ————. Untersuchungen über den Einfluss von Wurzelausscheidungen auf die Entwicklung von Bodenpilzen. Naturwiss. **45**: 63-64. 1958.

149. SCHREINER, O., and H. S. REED. The toxic action of certain organic plant constituents. Bot. Gaz. **45**: 73-102. 1908.

150. ————, and E. C. SHOREY. The isolation of picoline carboxylic acid from soils and its relation to soil fertility. Jour. Amer. Chem. Soc. **30**: 1295-1307. 1908.

151. ———— and ————. The isolation of dihydroxystearic acid from soils. Jour. Amer. Chem. Soc. **30**: 1599-1607. 1908.

152. SIGMUND W. Über die Einwirkung von Stoffwechselprodukten auf die Pflanzen II. Einwirkung N-freier pflanzlicher Stoffwechselprodukte auf die Keimung von Samen. (Glucoside, Gerbstoffe und ihre Spaltungsprodukte.) Biochem. Zeits. **62**: 339-386. 1914.

153. ————. Über die Einwirkung von Stoffwechsel-Endprodukten auf die Pflanzen. III. Einwirkung N-freier pflanzlicher Stoffwechsel-Endprodukte auf die Keimung von Samen: Ätherische Öle, Terpene u. a. Biochem. Zeits. **146**: 389-419. 1924.

154. SPICHER, G. Untersuchungen über die Wechselwirkungen zwischen *Azotobacter chroococcum* und höherer Pflanze. Zentr. Bakteriol. Parasitenk. Abt. II. **107**: 353-383. 1954.

155. STEINEGGER, E., and H. LEUPI. Influence of plant substances on the root growth of *Allium cepa* and germination of *Lepidium sativum*. I. Pharm. Acta Helv. **30**: 452-461. 1955. Chem. Abstr. **50**: 10194d. 1956.

156. STILLE, B. Schädigungen an Pflanzenwurzeln durch Kulturfiltrate von Mikroorganismen. Arch. Mikrobiol. **26**: 71-82. 1957.

157. STOLP, H. Beiträge zur Frage der Beziehungen zwischen Mikroorganismen und höheren Pflanzen. Arch. Mikrobiol. **17**: 1-29. 1952.

158. STOUT, M., and B. TOLMAN. Interference of ammonia released from sugar beet seed balls, with laboratory germination tests. Jour. Amer. Soc. Agron. **33**: 65-69. 1941.

159. ———— and ————. Factors affecting the germination of sugar-beet and other seeds, with special reference to the toxic effects of ammonia. Jour. Agr. Res. **63**: 687-713. 1941. Chem. Abstr. **36**: 2293-2294. 1942.

160. SWART-FÜCHTBAUER, H. Ektoparasitische Nematoden als mögliche Ursache der Bodenmüdigkeit in Baumschulen. Naturwiss. **41**: 148. 1954.

161. TOLLE, R., und A. RIPPEL-BALDES. Untersuchungen über die Rhizosphäre von Gramineen. Zentr. Bakteriol. Parasitenk. Abt. II. **111**: 204-217. 1958.

162. TOOLE, E. H., S. B. HENDRICKS, H. A. BORTHWICK, and V. K. TOOLE. Physiology of seed germination. Ann. Rev. Plant Physiol. **7**: 299-324. 1956.

163. ULLMAN, S. B. On germination inhibitors. V. Essential oils, alkaloids and glucosides as inhibitors of germination and growth. Ph. D. Thesis, Jerusalem, 1940. [After Evenari (52)]

164. USLAR, J. H. VON. Die Wurzeln der Pflanze oder die Bodenvergiftung durch die Wurzelausscheidungen der Pflanze. Hamburg. 1852.

165. VAN OVERBEEK, J., R. BLONDEAU, and V. HORNE. Trans- cinnamic acid as an anti-auxin. Amer. Jour. Bot. **38**: 589-595. 1951.

166. VARMA, S. C. On the nature of competition between plants in the early phases of their development. Ann. Bot. [London NS] **2**: 203-236. 1938.

167. VELDSTRA, H., and E. HAVINGA. On the physiological activity of unsaturated lactones. Enzymologia **11**: 373-380. 1945.

168. VIRTANEN, A. I., T. LAINE, and S. v. HAUSEN. Excretion of amino acids from the root nodules of leguminous plants. Nature **137**: 277. 1936.

169. WAKSMAN, S. A. Soil deterioration and soil conservation from the viewpoint of soil microbiology. Jour. Amer. Soc. Agron. **29**: 113-122. 1937.

170. WARD, G. M., and A. B. DURKEE. The peach replant problem in Ontario. III. Amygdalin content of peach tree tissues. Canad. Jour. Bot. **34**: 149-422. 1956.

171. WENSLEY, R. N. The peach replant problem in Ontario. IV. Fungi as-

sociated with replant failure and their importance in fumigated and nonfumigated soils. Canad. Jour. Bot. **34**: 967-981. 1956.

172. WENT, F. W. The dependence of certain annual plants on shrubs in Southern California deserts. Bull. Torrey Bot. Club **69**: 100-114. 1942.

173. WEST, P. M., and A. G. LOCHHEAD. Qualitative studies of soil microorganisms. IV. The rhizosphere in relation to the nutritive requirements of soil bacteria. Canad. Jour. Res. **18C**: 129-135. 1940.

174. WINTER, A. G. Untersuchungen über Vorkommen und Bedeutung von antimikrobiellen und antiphytotischen Substanzen in natürlichen Böden. Zeits. Pflanzenernähr. Düng. u. Bodenk. **69**: 224-233. 1955.

175. WINTER, A. G., und W. BUBLITZ. Über die keim- und entwichlungshemmende Wirkung der Buchenstreu. Naturwiss. **40**: 416. 1953.

176. ————, und F. Schönbeck. Untersuchungen über die Beeinflussung der Keimung und Entwicklung von Getreidesamen durch Kaltwasserauszüge aus Getreidestroh. Naturwiss. **40**: 168-169. 1953.

177. ———— und ————. Untersuchungen über den Einfluss von Kaltwasserextrakten aus Getreidestroh und anderer Blattstreu auf Wurzelbildung und -wachstum. Naturwiss. **40**: 513-514. 1953.

178. ———— und ————. Untersuchungen über wasserlösliche Hemmstoffe aus Getreideböden. Naturwiss. **41**: 145-146. 1954.

179. ————, und E. Sievers. Untersuchungen über die Beeinflussung der Samenkeimung durch Kaltwasserextrakte aus der Blattstreu verschiedener Gramineen. Naturwiss. **39**: 191-192. 1952.

180. ————, und L. Willeke. Untersuchungen über Antibiotica aus höheren Pflanzen und ihre Bedeutung für die Bodenmikrobiologie und Pflanzensoziologie. Naturwiss. **38**: 262-264. 1951.

181. WRENSHALL, C. L., and R. R. MCKIBBIN. Pasture studies. XII. The nature of organic phosphorus in soils. Canad. Jour. Res. B. **15**: 475-479. 1937.

182. YOSHIDA, R. K. Studies on organic phosphorus compounds in soil; isolation of inositol. Soil Sci. **50**: 81-89. 1940.

Future Contents of

THE BOTANICAL REVIEW

Articles Received and Awaiting Publication

Ecological Effects of Forest Fires	I. F. Ahlgren and C. E. Ahlgren, *Quetico-Superior Wilderness Research Center, Ely, Minnesota*
The Divisions and Classes of Plants	Arthur Cronquist, *New York Botanical Garden*
Cytology and Genetics of Forage Grasses..	H. L. Carnahan and Helen D. Hill, *U. S. Dept. of Agriculture*
The Climax Concept	G. D. Shelbck, *University of Saskatchewan*
Biological Antagonisms Due to Phytotoxic Root Exudates	Frank W. Woods, *Southern Forest Experiment Station*
The Lemnaceae	W. S. Hillman, *Yale University*
Histochemistry and Function of the Endodermis	D. S. Van Fleet, *University of Toronto*
Classification of Natural Communities	R. H. Whittaker, *Brooklyn College*
Physiology of Pollen	B. M. Johri & I. K. Vasil, *University of Delhi*
Differential Growth Inhibitors Produced by Plants	Solomon Garb, *Albany Medical College*
Genotypic Control of Chromosome Form and Behavior	H. Rees, *University of Birmingham, England*
Seedling Morphology in Applied Genetics and Plant Breeding	Gordon Haskell, *Scottish Hort. Res. Inst.*
Wild Plant Hybrid Frequencies	H. T. Clifford, *University of Queensland*
Chemical Tests in the Diagnosis of Plant Virus Diseases	R. C. Lindner, *Washington State University*
Techniques for the Observation and Isolation of Soil Microorganisms	R. D. Durbin, *University of Minnesota*

Comprehensive and up-to-date . . .

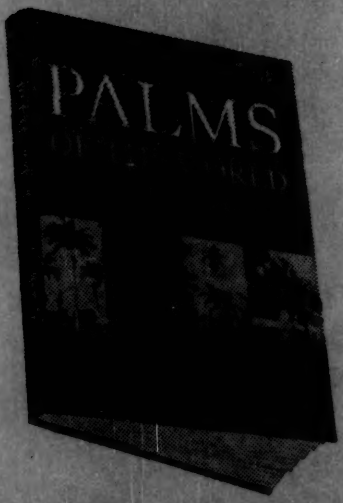
PALMS OF THE WORLD

by James C. McCurrach

This authoritative, beautifully produced and illustrated book covers alphabetically all known genera of palms: the distinctive characteristics of each, its reproduction, origin, and cultivation. Extensive data is given on the cultivation of species that can be grown in North America and the Caribbean, and the appendix provides complete lists of the species of palms grown at each of the main botanical gardens for this family in the United States.

In preparing this invaluable reference work, the author not only has drawn on the writings of the best-known authorities and on personal study of the living plants in many areas, but has consulted with botanists in North and South America, Europe, and Asia.

"Up to now there has been no single volume purporting to present an illustrated treatment of this great family of plants. The keen interest of James C. McCurrach, a plant collector of the first order, has fortunately brought such a book into being . . . The wealth of illustrations alone will serve as a guide to all enthusiasts interested in one of the green world's most fascinating groups of plants."—W. H. HODGE, President, The Palm Society.



ILLUSTRATED WITH MORE THAN 400 PHOTOGRAPHS

A BEAUTIFUL, OUTSIZE VOLUME WITH APPENDIX AND ILLUSTRATED GLOSSARY

At all bookstores • $17.50

HARPER & BROTHERS

New York 16